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HELMINTHS FROM THE ROBIN, WITH THE DESCRIPTION OF A NEW NEMATODE, *PORROCAECUM BREVISPICULUM*

J. Dan Webster*
Rice Institute, Houston, Texas

In the autumn of 1939 the writer examined thirteen robins (*Turdus migratorius*) taken near Ithaca, New York, mostly by Dr. Joseph C. Howell, who also contributed parasites and data from several other robins taken in the same locality in 1937. The following worms were found: one trematode, *Lyperosomum monenteron* Price and McIntosh, 1935 (Dicrocoeliidae); two cestodes, *Hymenolepis planestici* (Mayhew, 1925) Fuhrmann, 1932 (Hymenolepididae) and *Dilepis undula* (Schrank, 1788) Fuhrmann, 1908 (= *Southwellia ransomi* Chapin, 1926 according to Fuhrmann [1932: 100]) (Dilepididae); four nematodes, *Porrocaecum ensicaudatum* (Zeder, 1800) Baylis, 1920; *Porrocaecum brevispiculum* n. sp. (Ascarididae); *Acuaria* (*Dispharynx*) *spiralis* (Molin, 1858) Railliet, Henry, and Sissof, 1912 (Spiruridae); and *Syngamus trachea* (Montagu, 1811) Chapin, 1925 (Strongylidae); one acanthocephalan, *Plagiorhynchus formosus* Van Cleave, 1918 (Acanthocephalidae).

Lyperosomum monenteron, which has previously been reported from the bluebird in Virginia and the robin in Quebec, Virginia, and Washington, D. C., by Price and McIntosh (1935), and from the robin and mockingbird in Texas by Denton (1942), was found in three of thirteen robins in which the liver and bile ducts were examined. *Hymenolepis planestici* was found in four of nineteen birds and *Dilepis undula* in nine of nineteen.

Thirty-odd specimens of the genus *Porrocaecum* were taken from the intestines of seven of 19 robins examined, but specific identification was not made until after record was lost of the particular host of each individual worm. Five of these specimens—four males and one female—are assigned to *P. ensicaudatum;* the rest belong to a new species. These five individuals all lack lateral alae and otherwise agree with Baylis' (1922) diagnosis of *P. ensicaudatum,* save that two males had very long spicules that necessitate using Lewis' (1926) emendation—i.e., a greater maximum spicule length than the 620 to 630 μ designated by Baylis. Lewis' other emendation—occasional presence of lateral alae in *P. ensicaudatum*—is evidently applicable to North American material, because at least one of the five specimens from the Leidy Collection identified by Walton (1927: 63) as of this species posses-

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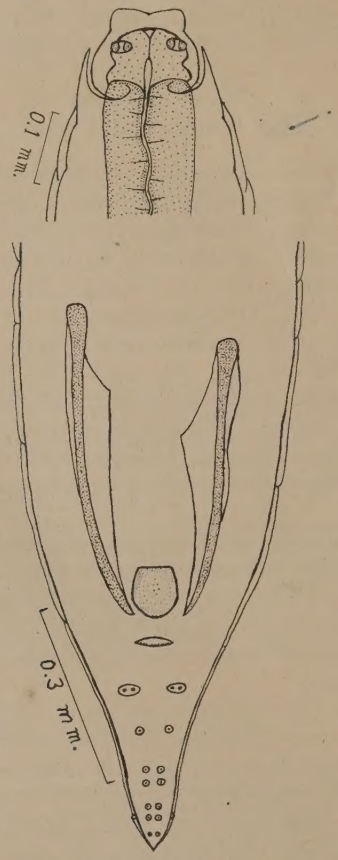
ses lateral alae. The writer concurs in this identification. The Leidy specimens were from the mockingbird, but *P. ensicaudatum* has previously been listed from the robin by Cram (1932) and Rayner (1932).

Porrocaecum brevispiculum n. sp.

(Figs. 1 and 2)

Diagnosis. Porrocaecum: Lateral alae present, little wider on neck than anywhere else. Each lip with a deep groove on either side of base, and a dentigerous ridge on anterior end. All



Porrocaecum brevispiculum n. sp.

FIG. 1 (upper). Dorsal aspect of lips of male. FIG. 2 (lower). Tail of male showing alate spicules, gubernaculum, and postanal papillae.

twelve external cephalic papillae well developed; incompletely fused in pairs. Three slender interlabia extend to just half length of ventriculus.

Males. Seven specimens measured 18 to 30 (av. 23.8) mm long and 526 to 794 (av. 611) μ wide. Dorsal lip 179 to 198 (av. 193) μ wide. Esophagus 1.53 to 2.10 (av. 1.69) mm long or 6 to 7% of body length. Ventriculus 313 to 632 (av. 398) μ long; cecum 22 to 124 (av. 88) μ long. Seven or eight pairs of postanal papillae present; one pair, doubles, just posterior to anus, one pair laterally very near tail, five or six pairs ventrally. Ventral pair of papillae immediately anterior to lateral pair sometimes absent. A barrel-shaped gubernaculum present, 85 to 190 μ long, placed between spicules, immediately anterior to anus, and partially surrounded by spicular

alae. Alate spicules subequal, left longer, 346 to 567 (av. 470) μ and right shorter, 256 to 487 (av. 408) μ. Tail, measured from posterior border of anus to tip, 208 to 471 (av. 295) μ long.

Females. Six specimens measured 28 to 42 (av. 36) mm long and at vulva 612 to 727 (av. 671) μ wide. Dorsal lip 225 to 249 (av. 233) μ wide. Esophagus 1.55 to 2.66 (av. 2.01) mm long or 5 to 7% of body length. Ventriculus 403 to 502 (av. 417) μ long. In one case cecum slightly exceeded half length of ventriculus. Tail 583 to 809 (av. 659) μ long, relatively thick and blunt as compared with male tail. Vulva somewhat posterior to junction of first and second thirds of body, dividing it in ratio of from 1/2.1 to 1/2.6. Eggs ellipsoid; outer shell with cobble-stone-like surface paved with irregularly rounded plates; eggs 81.1 to 96.5 (av. 91.3) μ by 56.0 to 64.3 (av. 60.9) μ.

Host: Turdus migratorius.

Habitat: Small intestine.

Locality: Ithaca, New York.

Type: Male, U. S. N. M. Helm. Coll. No. 40687; paratype—female, U. S. N. M. Helm. Coll. No. 40687.

Porrocaecum brevispiculum is distinguishable from the closely related *P. cheni* Hsü, 1933, by the presence in the former of lateral alae, a gubernaculum, shorter spicules, and a more anteriorly-placed vulva. The other species with a rudimentary intestinal cecum, *P. ensicaudatum,* lacks a gubernaculum, and possesses lateral wings on the pulp of each lip and longer spicules than are found in *P. brevispiculum.* Possibly the immature *Porrocaecum* which Chapin (1926) mentions taking from a Washington, D. C., robin were also of this species.

Acuaria spiralis was found in the stomachs of five of 19 robins; in one case about 150 worms had eroded most of the proventricular mucosa. A single *Syngamus trachea* was found in the nineteen robins examined.

Dr. Howell found three robins, each of which was dying, apparently from the pathogenic effects of one or two acanthocephalans, identified as *Plagiorhynchus formosus.* Pathogenic effects of this parasite upon the robin have previously been reported by Jones (1928).

References

Baylis, H. A. 1922 Note on two species of *Porrocaecum* from birds. Ann. and Mag. Nat. Hist. **9**: 499–504.

Chapin, E. A. 1926 *Southwellia ransomi* new species. J. Parasitol. **13**: 29–33.

Cram, E. B. 1932 New records of nematodes of birds. J. Parasitol. **19**: 93.

Denton, J. F. 1943. A revision of the trematode subfamily Dicrocoeliinae with descriptions of new species from North American hosts. Proc. U. S. Nat. Mus. (In press.)

Fuhrmann, O. 1932 Les Tenias des Oiseaux. Mem. Univ. Neuchatel. **8**: 1–381.

Hsü, H. F. 1933 Some species of *Porrocaecum* (*Nematoda*) from birds in China. J. Parasitol. **19**: 280–287.

Jones, M. 1928 An acanthocephalid, *Plagiorhynchus formosus,* from the chicken and the robin. J. Agric. Research, U. S. Dept. Agric. **36**: 773–775.

Lewis, E. A. 1926 Helminths of wild birds found in the Aberytswyth area. J. Helm. **9**: 7–12.

Price, E. W. and McIntosh, A. 1935 A new trematode, *Lyperosomum monenteron* n. sp., from a robin. Proc. Helm. Soc., Washington **2**: 63–64.

Rayner, J. A. 1932 Parasites of wild birds in Quebec. Scient. Agric. (Rev. Agron. Canad.) **12**: 307–309.

Walton, A. C. 1927 A revision of the nematodes of the Leidy collection. Proc. Acad. Nat. Sc. Phila. **79**: 49–164.

THE DEVELOPMENT OF THE SPOROCYSTS OF A SCHISTOSOME, *CERCARIA STAGNICOLAE* TALBOT, 1936[1]

W. W. Cort and Louis Olivier[2]

During the summers of 1940 and 1941 at the University of Michigan Biological Station we made a study of the mother and daughter sporocysts of *Cercaria stagnicolae* Talbot, 1936, from natural infections in *Stagnicola emarginata angulata* (Sowerby). *C. stagnicolae* is the commonest schistosome in the Douglas Lake Region and is the most important cause of swimmers' itch in Michigan (McMullen and Brackett, 1941). Large numbers of infections of this cercaria come to maturity in adult snails in late June and early July; on the other hand, infections are very rare in juvenile snails from the time they begin to hatch in early June through the first third of September (Cort et al, 1937; Cort et al, 1940). Therefore, it was concluded that the snails acquire most of their infections with *C. stagnicolae* late in the fall and early in the spring. Thus, the examination of adult snails in May and June would offer the best opportunity to study the early development of the sporocysts of this species. Our studies were actually made from June 26 to July 11, 1940, and from June 20 to July 3, 1941. Even this late we found numerous mother sporocysts, some still immature, and followed the early development of the daughter sporocysts; but as was to be expected very early stages of mother sporocysts were not found.

The methods used in this study were the same as those employed in our earlier investigations (Cort and Olivier, 1941). *C. stagnicolae* is the only schistosome that has ever been found in *S. e. angulata* in the region with the exception of a single infection of *Schistosomatium douthitti* (Cort et al, 1937). Schistosome mother and daughter sporocysts are easy to differentiate from those of strigeids and plagiorchiids; and after we had once determined the position of the mother sporocysts in the snails they were not difficult to find. In all, we obtained more than fifty mother sporocysts of this species and were able to make more or less detailed observations on more than thirty of them. About half of these were considered to be mature since they contained daughter sporocysts that appeared ready to emerge. We were also able to obtain for study a number of immature daughter sporocysts.

MOTHER SPOROCYSTS OF *C. stagnicolae*

The mother sporocysts of *C. stagnicolae* are very thin walled, sausage shaped sacs, which, even when they contain numerous well developed daughter sporocyst embryos, appear quite empty, with the germinal material filling only a fraction of the inflated lumen. The youngest that were found were about 3 mm in length and from 0.10 to 0.17 mm in width, and the largest were over 10 mm long and about 0.2

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[1] From the University of Michigan Biological Station and the Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University.

This is one of a series of investigations on the early developmental stages of digenetic trematodes in the snail hosts. Previous studies have been on strigeids (Cort and Olivier, 1941) and a plagiorchiid (Cort and Olivier, 1943).

[2] Junior Zoologist, Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville Experimental Center, Beltsville, Md.

to 0.3 mm broad. These measurements of width are probably too small because freed sporocysts whether broken or not usually became more or less deflated.

Most of the mother sporocysts were located on the surface of the kidney and stomach of the snails; others were found on the surface of the uterus and nidamental gland, along the intestine, and in the proximal part of the digestive gland. In all cases they were more or less firmly laced for a part of their length into the connective tissue covering the organs; and when the snails were removed from their shells for examination portions of the mother sporocysts could frequently be seen sticking out from the surfaces of the organs. Both when in position in the tissues of the host and when placed in the saline solution for study their power of movement was very limited, their activity being very much less than that of the thicker walled mother sporocysts of strigeids. One end is more attenuated than the other and except in very immature specimens a birth pore and canal can be clearly seen (Fig. 1).

The body wall of the mother sporocysts of *C. stagnicolae* has a very thin cuticula and a layer containing flattened stellate cells with rather small nuclei. Circular and longitudinal muscle elements must be present but could not be seen in living material. The cellular layer is thicker and its nuclei are more numerous in a given area in the younger as compared with the older sporocysts. The stellate shape of the cells of this layer can be seen best in surface view as shown in Fig. 2. In the younger mother sporocysts the wall is colorless and translucent, but as they grow older it becomes more and more greyish in appearance from the accumulation in the cellular layer of minute, oily droplets. Few flame cells were ever seen in the wall, whereas in strigeid mother sporocysts flame cells are numerous and very conspicuous. In the youngest mother sporocysts of *C. stagnicolae* the body cavity was traversed by numerous protoplasmic processes; but in the older ones these processes were absent and the contents moved back and forth freely.

In the smallest mother sporocysts most of the germinal material was attached to the protoplasmic processes which extended from the body wall. It consisted of single germ cells, groups of a few germ cells, germ balls which appeared to be very young daughter sporocyst embryos, and germ masses with both unicellular and multicellular components. There were comparatively few isolated germ cells, due probably to the fact that our earliest stages were about 3 mm in length. In one of the youngest mother sporocysts studied, however, there were regions where only single germ cells were present attached to the processes that traversed the cavity (Fig. 3). Fig. 4 shows a part of another very immature sporocyst showing single germ cells, irregular groups of three to several germ cells, and some germ balls. In two other very immature mothers there were groups composed of small germ balls and germ cells, which were held in the lumen of the cavity by the processes from the wall. These groups of germinal material, which may be considered to be "germ masses," were separated by empty spaces not traversed by the processes from the wall. They were held firmly in position and did not move back and forth in the lumen. Fig. 5 shows the posterior end of one of these two sporocysts in which the position and structure of five such "germ masses" are shown.

In what appeared to be a slightly older mother sporocyst there was present a number of oval daughter sporocyst embryos floating free in the cavity with no strands connecting them with the wall. In a few places in this sporocyst, however, strands could be seen with single germ cells and small groups of germ cells attached to them (Fig. 6).

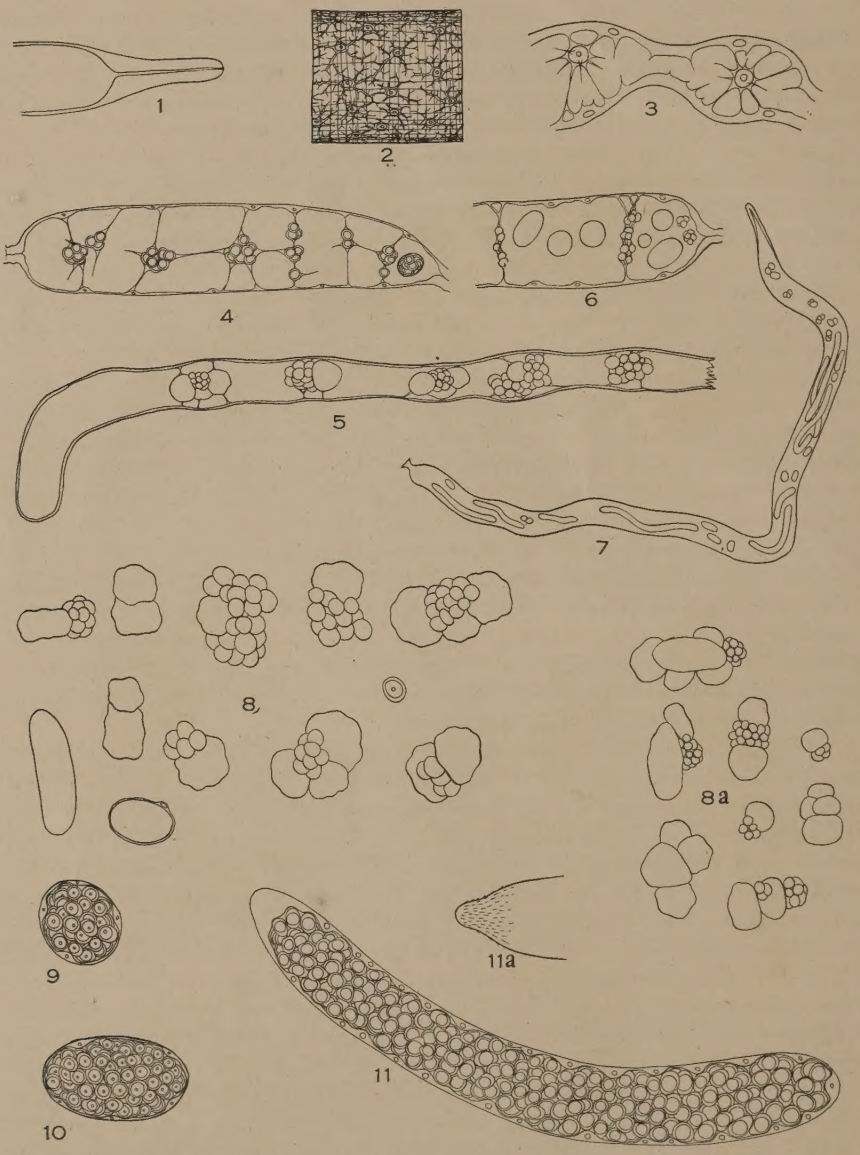

FIG. 1. Anterior end of immature mother sporocyst showing birth pore and canal.

FIG. 2. Surface view of wall of mother sporocyst showing the characteristics of the cells.

FIG. 3. A small section of a very immature mother sporocyst showing germ cells suspended by strands from the wall.

FIG. 4. Section of immature mother sporocyst showing single germ cells and cell groups suspended by strands from the wall.

FIG. 5. Posterior end of immature mother sporocyst showing 5 germ masses attached by strands to the wall.

A somewhat later stage was represented by another immature mother sporocyst that was freed unbroken and mounted whole for study. Two of its daughter sporocyst embryos had become somewhat elongate; the others were in earlier developmental stages. Its anterior end was attenuated and the birth pore and canal could be made out. Close to this end there was a group of a few germ cells held by strands from the wall. A little further along there was an irregular germ mass and beyond this several small germ balls (daughter sporocyst embryos), also, attached to the wall. Throughout the rest of the length of this sporocyst no further processes crossing the lumen were present and the contents which consisted of numerous daughter sporocyst embryos floated freely back and forth. In all over 60 embryos were counted in this mother.

Mother sporocysts were considered to have reached maturity when they contained fully developed daughter sporocyst embryos that moved actively and appeared ready to escape (Fig. 7). In some infections with mature mother sporocysts, immature daughters that had already escaped into the snail tissues were present. There were even a few cases in which old mother sporocysts still containing a few embryos persisted when some of their daughter sporocyst brood had become mature and contained fully developed cercariae. As already noted the mature mother sporocysts were larger and thinner walled than the immature ones and appeared greyish. The sporocyst cavity was not traversed by strands and its contents, which only filled a fraction of the lumen, moved freely back and forth. All stages of daughter sporocyst embryos were present in mature mother sporocysts including elongate active forms that appeared ready to escape. In addition, there were also present a few irregular masses of germinal material, some of which included both germ balls and single cells, and others only germ balls. These germ masses were irregular in shape, varied greatly in size and composition, and did not seem to have a membrane of their own. Figs. 8 and 8a show a series of them from several different mature mother sporocysts. These free floating germ masses of the mature mother sporocysts, which, as noted above, are also found in immature mothers, are like the masses found attached to the protoplasmic strands in some of the younger sporocysts (Fig. 5). They probably are masses of the same type which have been freed from the strands connecting them with the wall. Usually, also, a few isolated germ cells were found free in the lumen of mature mother sporocysts. These may have broken loose from floating germ masses.

In a few cases it was possible to make counts of the germinal elements present in mature mother sporocysts. One complete, large, well developed mother sporocyst contained 6 motile, elongate daughter sporocysts which appeared about ready to escape, 13 elongate embryos, 11 that were large and oval, and over 40 earlier

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Fig. 6. Section of immature mother sporocyst showing germinal material on strands from body wall and free germ balls (daughter sporocyst embryos).

Fig. 7. Anterior portion of mature mother sporocyst, showing daughter sporocyst embryos in different stages of development and germ masses.

Fig. 8. Series of germ masses and two daughter sporocyst embryos for size comparison.

Fig. 8a. Germ masses from mother sporocysts drawn to smaller scale.

Fig. 9. Daughter sporocyst embryo in germ ball stage. Diameter 0.049 mm.

Fig. 10. Daughter sporocyst embryo beginning to elongate. Length, 0.094 mm.

Fig. 11. Daughter sporocyst ready to escape from mother (migrating stage). Length, 0.371 mm; width, 0.051 mm. Specimens from which this and two previous drawings were drawn were somewhat flattened.

Fig. 11a. Anterior tip of daughter sporocyst showing spines.

embryos in the germ ball stage. There were, also, a few germ masses, some of which contained both multicellular and unicellular components. No separate germ cells were seen in this particular sporocyst. It appeared to have just reached maturity since a careful search revealed no developing daughter sporocysts in the snail's tissues. In another mature mother sporocyst there were over 100 daughter sporocyst embryos in all stages of development and six germ masses. Another contained over 25 elongate, motile daughter sporocysts and a number of younger embryos at various stages. A part (length 4.2 mm) of an old mother sporocyst had 9 elongate, motile daughter sporocysts, 15 younger developing embryos, and a single germ mass consisting of one germ ball to which several single cells were attached. In this infection developing daughter sporocysts, the oldest of which was almost mature, were found in the digestive gland of the snail. In another old mother sporocyst, some of whose daughters had emerged and reached maturity, there were still 5 fully developed daughter sporocyst embryos, 28 others in all stages of development, and 8 "germ masses" of which half still showed single germ cells. In most of the old mother sporocysts, however, the amount of embryonic material was much less.

STAGES OF DEVELOPMENT OF DAUGHTER SPOROCYST EMBRYOS

The smallest daughter sporocyst embryos within the mothers were small, spherical germ balls which were just like the larger multicellular components of the germ masses. In some of the larger of these germ balls it was possible to see a thin, outer membrane; and in some, about 0.02 to 0.03 mm in diameter, differentiated, flattened cells of the enclosing membrane could occasionally be made out. When embryos 0.04 to 0.05 mm in diameter were stained lightly with neutral red, it was possible to see clearly the relation of the wall and contents (Fig. 9). Such embryos consisted of a group of large distinctly separate germ cells, enclosed by a thin wall composed of flattened cells, with small nuclei and coarsely granular, vacuolated cytoplasm. The germ cells had large, clear nuclei, conspicuous nucleoli, and a thin, outer layer of homogeneous, finely granular cytoplasm. They were like the germ cells found free or in germ masses in the mother sporocysts. Somewhat larger embryos, 0.09 to 0.10 mm in length and 0.06 to 0.07 mm in width, had the same structure except that the germ cells were more numerous (Fig. 10). The structure was, also, essentially the same in older, elongate daughter sporocyst embryos, which were from 0.25 to 0.30 mm in length and 0.03 to 0.04 mm in width. In them the body wall was thicker and much more differentiated and the number of germ cells in the cavity was considerably larger.

The motile daughter sporocysts (migrating stage) which appeared ready to escape from the mother were studied carefully using neutral red as an *intra vitam* stain (Fig. 11). They moved actively and extended and contracted the body considerably. A series of ten from one mother sporocyst, measured alive had an average size of 0.37 by 0.05 mm, and the largest seen was 0.64 mm in length. Their anterior end is mobile and tapering and bears numerous small spines (Fig. 11a). They have a thin cuticula and well developed muscle layers. No spines except those on the anterior end were present and no cilia could be seen on their surface, even when they were studied with dark field illumination at magnifications up to 1000 times. In this respect they differ from the description given by Price (1931) for the immature daughter sporocysts of *S. douthitti*. The body cavity of these daughter

sporocysts is crowded with large numbers of germ cells (over 100 counted in one case) which are not attached to each other or to the body wall. These germ cells are tightly packed into the cavity and completely fill it for its whole length; no multicellular groups were ever seen at this stage. Each daughter sporocyst has two terminal, posterior excretory pores. It seems probable that normally the daughter sporocysts escape only by way of the birth pore of the mother, and in one case one was seen in the act of escaping (Fig. 12).

DEVELOPMENT OF THE DAUGHTER SPOROCYSTS OUTSIDE THE MOTHER

After leaving the mother, most of the daughter sporocysts migrate into the digestive gland of the snail, although they may become located on the surface of the organs in front of this gland. They penetrate into all parts of the parenchyma of the digestive gland and develop in intimate contact with the tissues. When found among the organs in front of the digestive gland they are also in the connective tissues. Since the daughter sporocysts are so thin walled and are in such intimate contact with the snail's tissues, it proved very difficult to free them unbroken, especially after they had increased considerably in size.

The daughter sporocysts that have just escaped from the mother sporocyst have the same structure as that described above for those still in the mother. Their great mobility and probably the spines at the anterior end make migration possible. Since these migrating daughter sporocysts grow directly into the large mature daughter sporocysts it is obvious that they increase greatly in size. Also, there must be some method of multiplication of their germ cells, since, from what we know of cercarial production of this schistosome infection, it seems clear that there are not enough germ cells present in a migrating daughter, if each developed into one cercaria, to account for the large numbers of cercariae produced.

Some very early stages in the development of the free daughter sporocysts were available for study. One of the youngest found, which was about 0.5 mm in length is shown in Fig. 13. It can be seen that for about the anterior one-third this sporocyst contained no germinal material, and showed strands of tissue traversing the body cavity. The middle third was only partly filled with germinal material, most of which consisted of single cells with only a few multicellular groups. The posterior third was crowded with separate germ cells.

Another immature daughter sporocyst of about the same stage of development is shown in Fig. 14. Its body cavity contained large numbers of single germ cells, but there were about 14 multicellular groups. Some of these groups were made up of only two to four cells, but several had considerably larger numbers. Each group evidently came from the division of one of the single germ cells, but it could not be determined whether they were developing directly into cercarial embryos or would become germ masses. The whole body cavity of this sporocyst was completely filled with germinal material except the posterior end and there were no protoplasmic processes traversing the lumen.

Only the posterior end of another very young daughter sporocyst was obtained for study (Fig. 15). In this specimen the body cavity was traversed by protoplasmic strands on which were held both single germ cells and multicellular groups.

A portion of another very immature daughter sporocyst was also studied (Fig. 16). In it the germinal material only filled a fraction of the body cavity, which was traversed by numerous protoplasmic strands. Held on these strands were numerous

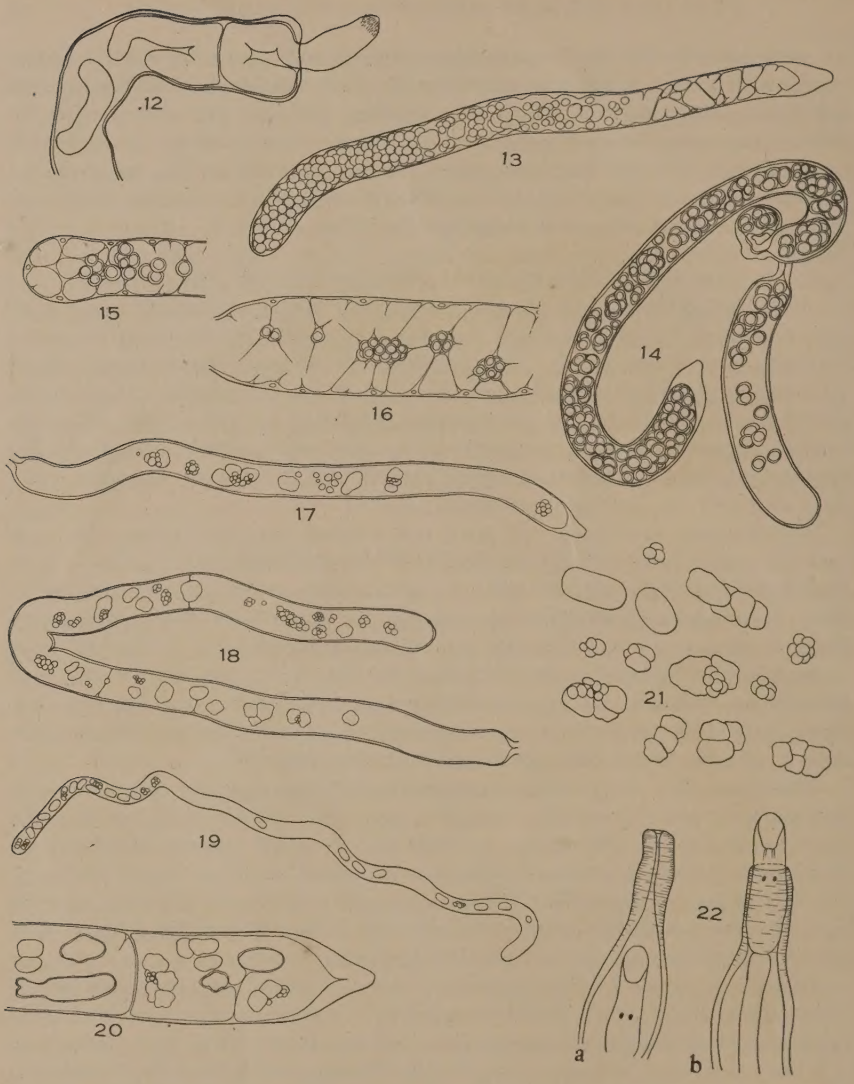

FIG. 12. Daughter sporocyst escaping through birth pore of mother. Specimen much flattened.

FIG. 13. Early stage of daughter sporocyst soon after escape from mother. Length, 0.65 mm.

FIG. 14. Very immature daughter sporocyst after escape from mother sporocyst.

FIGS. 15 and 16. Parts of immature daughter sporocyst showing attachment of germinal material to wall by strands.

FIGS. 17 and 18. Pieces of immature daughter sporocysts with germinal material floating free in body cavity.

FIG. 19. Immature daughter sporocyst in which cercarial embryos are beginning to show differentiation.

FIG. 20. Anterior end of slightly older daughter sporocyst in which oldest embryo shows differentiation into body and tail.

FIG. 21. Germ masses from daughter sporocysts. Two daughter sporocyst embryos included for size comparison.

FIG. 22 a and b. Anterior ends of mature daughter sporocysts showing birth pore and canal.

separate germ cells, some early cleavage stages of two to four cells, and a few groups of up to 16 cells. The largest cell groups did not look like cercarial embryos since the cells in them were only loosely held together.

Several other very immature daughter sporocysts in which the body cavity was traversed by protoplasmic strands bearing the germinal material were also observed. In all these sporocysts most of the lumen was empty. Such sporocysts usually contained some irregular germ masses. Immature sporocysts were also found in which there were very few protoplasmic strands traversing the body cavity and which had free in the lumen germinal material consisting of single germ cells, groups of a few cells, and irregular germ masses composed of both unicellular and multicellular components (Figs. 17 and 18).

Daughter sporocysts that appeared to be in later stages of development contained numbers of structures that could be definitely recognized as cercarial embryos. One such sporocyst which was removed unbroken is shown in Fig. 19. It measured 4.5 mm in length and 0.13 mm in width. No protoplasmic strands could be seen crossing the body cavity. It contained a surprisingly small amount of germinal material, since its total contents consisted of 7 irregular germ masses 0.040 to 0.050 mm in diameter and 18 cercarial embryos 0.050 to 0.075 mm in length, none of which showed differentiation into body and tail. Another immature daughter sporocyst that was freed intact contained no single germ cells, about 25 to 30 germ masses and a few small cercarial embryos. The small amount of germinal material in these two sporocysts was rather surprising and appeared to be much less than in most of the immature daughter sporocysts seen.

A number of daughter sporocysts were observed at a somewhat older stage when the oldest cercarial embryos were just beginning to show differentiation into body and tail. These, also, contained numerous cercarial embryos in the germ ball stage and a varying number of germ masses. In only a few cases were there any protoplasmic strands in the body cavity and no separate germ cells were seen. Fig. 20 shows the anterior end of a sporocyst in this stage which still retained a few protoplasmic strands.

A number of daughter sporocysts approaching maturity were also studied. In general, most of their contents consisted of immature cercariae at approximately the same stage of development, which filled most of the cavity. In most of these sporocysts small numbers of young embryos and a few germ masses were also present. Even in sporocysts that contained mature cercariae germ masses and very young embryos were sometimes found. Frequently, however, only fairly well developed cercariae were present in mature sporocysts.

The germ masses of the daughter sporocysts of *C. stagnicolae* are similar to those of the mother. A series of them from several different sporocysts of different ages is shown in Fig. 21. Many of them have only multicellular components, usually varying in number from two to five; but others are made up of both multicellular and unicellular components. They vary greatly in shape, size, and number of components, and do not seem to have a membrane surrounding them. The largest multicellular components of the germ masses correspond in structure to the smallest cercarial embryos (Fig. 21).

Mature daughter sporocysts have a thin, fragile wall which contains varying amounts of minute, refractile, oily droplets giving them a more or less opaque ap-

pearance. It appears that the older the sporocysts are, the more numerous are the globules in the wall so that in old infections the sporocysts appear quite black in transmitted light. Mature sporocysts are almost completely non-motile, except as influenced by the movements of the cercariae that they contain and are tightly laced into the tissue of the snail. It was very difficult to free them for study; in fact, in no cases were we able to isolate a complete one. In some infections producing mature cercariae some of the sporocysts contained only immature cercariae. Often when all the sporocysts had reached maturity there would also be present varying numbers of partially or entirely empty sporocysts which had collapsed and were degenerating.

Mature daughter sporocysts have an attenuated anterior end with a birth pore and canal, which are very conspicuous when cercariae are emerging (Figs. 22 a and b). The best way to see the birth pore is to pull the body of a snail containing a mature infection out of the shell, and observe the surface of the digestive gland in water or saline solution. In such preparations the anterior ends of numerous sporocysts may be seen projecting out from the surface of the digestive gland. In a short time cercariae will begin to emerge from the birth pores at the free ends. Several cercariae have been observed to emerge in a few minutes from the free end of a single sporocyst.

DISCUSSION

Although our picture is not complete, we can give a general outline of the probable development of the sporocysts and the course of the germ cell cycle of *C. stagnicolae*. Postulating that the miracidium of this species is like those of other schistosomes, it can be suggested that its body cavity contains a considerable number of germ cells attached to the wall by protoplasmic processes (Price, 1931, Pl. 2, Fig. 13; Tang, 1938, Pl. II; Wall, 1941, Fig. 19). In the early stages of the metamorphosis of the miracidium into the mother sporocyst a very rapid and extensive growth of the body wall takes place to produce the elongate sacs, which are the earliest mother sporocysts which we found. In these, germinal development is far outstripped by somatic growth since the germinal material only fills a small fraction of the body cavity. Since we found in our youngest stages separate germ cells in the cavity suspended by strands from the body wall, it appears that as the miracidium metamorphoses into an inflated elongate sac, its germ cells become distributed throughout the cavity by the extension of the strands which connect them with the body wall. Some of these germ cells may possibly develop directly into daughter sporocyst embryos without any multiplication, and others may divide into a few germ cells which later separate to form embryos. However, the finding of irregular, rather loosely organized germ masses, which in the younger stages are attached to the body wall by strands and which in later stages float free in the cavity, suggests that a phase of multiplication by polyembryony is commonly present. It seems probable that each of these germ masses is the product of a single germ cell. If, when a germ cell divided, the daughter cells remained loosely attached to each other and later one by one started to develop into embryos, the result would be the production of germ masses containing both multicellular and unicellular components like those shown in Figs. 8 and 8a. Then, after all the germ cells of a germ mass had started to develop into embryos, it would come to be composed only of multicellular components, which would break away one at a time until the mass was entirely disintegrated. The terminal stages would, therefore, be germ masses containing several or finally only two multicellular

components (Fig. 8). As more and more embryos became free in the cavity and as some of them developed into elongate, active daughter sporocysts, their movements, both passive and active, would tend to break down the protoplasmic strands across the lumen, and soon all the germinal material would float free in the cavity. Thus, germ masses would be found free in the cavity along with embryos in various stages of development.

There must be a considerable lag in the development of some of the germ masses of the mother sporocysts since a few are still present in mature and even old sporocysts. This lag would make possible the production of new daughter sporocysts throughout the whole reproductive life of the mother. In fact, a few very immature embryos were still present in old mother sporocysts even when the first daughters that had escaped already contained fully developed cercariae. However, the life of the mother sporocysts in *C. stagnicolae* appears to be relatively much shorter than in the strigeids since it is rare to find mother sporocysts in mature infections.

In the development of the daughter sporocyst embryos inside the mother there is a rapid multiplication of germ cells. In very early stages (germ balls) a few cells are differentiated which form the wall of the sporocyst and all the others go on multiplying until at the time of emergence from the mother the daughter sporocyst contains over a hundred separate germ cells which completely fill the body cavity (note stages of development in Figs. 9, 10, and 11). In spite of the large number of separate germ cells in the migrating stage of the daughter sporocysts of *C. stagnicolae* there must be further multiplication of germinal material to produce the large numbers of cercariae that develop. Consequently, as in the mother sporocyst, a phase of multiplication by polyembryony is introduced. After emergence from the mother the somatic development of the daughter sporocyst outstrips the germinal, since a stage is produced in which the germinal material fills only a fraction of the cavity. Protoplasmic strands are present extending from the wall across the lumen and the germinal material is attached to these strands. Just how this relation develops is not clear, but the fact remains that in immature daughter sporocysts germ cells, cleavage stages, germ masses and even separate embryos are frequently found attached to the walls by such strands.

Germ masses are so numerous and consistently present in immature daughter sporocysts of *C. stagnicolae* that it seems probable that all the numerous germ cells present in the daughter at emergence go through this type of multiplication. Although a few germ masses and early embryos are still present in daughter sporocysts in which most of the cercarial embryos are approaching maturity, many of the germ masses develop synchronously and a large proportion of the cercarial embryos in an older daughter sporocyst are at about the same stage of development. Consequently, in infections from which large numbers of cercariae are escaping a large proportion of the sporocysts will contain only mature or almost mature cercariae. This tendency toward synchronicity in the polyembryony in the daughter sporocysts leads to the development of large numbers of cercariae over a rather limited period of time and a rather rapid exhaustion of the infection.

In the sporocyst stages of *C. stagnicolae,* therefore, an enormous multiplication of individuals is brought about both by a multiplication of germ cells in the embryonic development of the daughter sporocysts and by polyembryony by means of germ masses in both the mother and daughter sporocyst stages. A comparison of the germ cell cycle of *C. stagnicolae* with that of the strigeids (Cort and Olivier, 1941) will

make these relations clearer. In the strigeids small numbers of germ cells in the miracidium produce small numbers of germ masses in the mother sporocysts. These germ masses are discrete entities with their own membrane, which persist throughout the whole life of the mother sporocysts and produce daughter sporocyst embryos by the breaking off of their largest end components. Even in old mother sporocysts, which persist in infections in which most of the daughter sporocysts are mature, the germ masses show no signs of reproductive exhaustion and contain numerous unicellular components. Also, the daughter sporocysts of strigeids, when ready to emerge, contain only germ masses (about 12 to 24). In the migrating stage these germ masses grow larger and begin to give off cercarial embryos. No separate germ cells are present. These germ masses persist as discrete entities and produce cercarial embryos throughout the life of the daughter sporocysts. This means that in the strigeids a constant supply of cercarial embryos is produced and that new cercariae are developing and escaping at a relatively constant rate throughout the whole life of the infection. It can be seen, therefore, that the germ cell cycle of *C. stagnicolae* differs chiefly in two particulars from that of the strigeids. In the first place its germ masses are not such discrete entities and the phases of multiplication by polyembryony, both in the mother and daughter sporocysts, seem to be more transient and irregular. In the second place, large numbers of separate germ cells are produced, especially in the development of the daughter sporocysts, before the germ masses appear.

In the few other descriptions of schistosome mother sporocysts we have found in the literature (Miyairi and Suzuki, 1914; Faust and Meleney, 1924; Price, 1931; and Wall, 1941) the contents of the body cavity are described as consisting of germ cells and early embryos in the germ ball stage. No structures that could be interpreted as germ masses were described or figured. In fact, in none of these accounts is the question of multiplication of the germinal elements even considered. Also, in the descriptions or figures of the early embryonic stages of the daughter sporocysts within the mother and of the immature stages in the snail's tissues accurate details are not provided. It seems clear, however, that the presence of spines on the anterior tip of young daughter sporocysts is probably a characteristic of the whole schistosome group since they have been described for *Schistosoma japonicum* by Miyairi and Suzuki (1914), for *S. mansoni* by Faust and Hoffman (1934), for *Schistosomatium douthitti* by Price (1931), and for *Spirorchis parvus* by Wall (1941). The birth pore of mother and daughter sporocysts has apparently not been noted for other schistosome species, although it seems probable that it is present. Certainly, on account of the lack of information on other schistosome species no generalizations on early sporocyst development and the course of the germ cell cycle can be made at the present time for the whole schistosome group.

SUMMARY

Studies were made from natural infections in *Stagnicola emarginata angulata* (Sowerby) of the mother sporocysts and the development of the daughter sporocysts of *Cercaria stagnicolae* Talbot, 1936. The mother sporocysts of this schistosome are thin walled, sausage shaped sacs with an attenuated anterior end bearing a birth pore and canal. They are usually located in the tissues on the surface of the organs of the snail in front of the digestive gland, particularly on the kidney and stomach. In the

smallest mother sporocysts found, which were about 3 mm in length, the germinal material, consisting of single germ cells, groups of a few germ cells, irregular germ masses, and a few germ balls (immature daughter sporocyst embryos), is attached to protoplasmic processes from the body wall. In somewhat older sporocysts the contents, consisting of germ masses and daughter sporocyst embryos, float freely in the body cavity and few, if any, protoplasmic processes persist. The irregular germ masses, some with only multicellular components and others with both multicellular and unicellular components, frequently are still present in mature mother sporocysts. The developing daughter sporocyst embryos consist of masses of separate germ cells enclosed by thin walls of flattened cells. Even in the daughter sporocysts that are ready to escape from the mother germinal material consists only of numerous separate germ cells.

After the escape of the daughter sporocysts into the tissues of the snail there is a rapid increase in size and a stage when the germinal material fills only a fraction of the body cavity and is attached to protoplasmic processes from the wall. Numerous irregular germ masses are produced similar to those found in the mother sporocysts. In stages of daughter sporocysts approaching maturity the contents consist of large numbers of cercarial embryos which nearly fill the cavity and few, if any, germ masses persist.

In the sporocyst stages of *C. stagnicolae*, therefore, enormous cercarial production is made possible by the large numbers of germ cells that develop in the daughter sporocysts before they leave the mother and by phases of polyembryony by germ masses in both the mother and daughter sporocysts. The germ cell cycle of *C. stagnicolae* differs from that of the strigeids chiefly in two particulars, viz., (1) its germ masses are not such discrete entities and the phases of polyembryony, both in the mother and daughter sporocysts, appear to be more transient and irregular, and (2) large numbers of separate germ cells are produced, especially in the development of the daughter sporocysts, before the germ masses appear.

References

Cort, W. W., McMullen, D. B. and Brackett, Sterling 1937 Ecological studies on the cercariae of *Stagnicola emarginata angulata* (Sowerby) in the Douglas Lake region, Michigan. J. Parasitol. **23**: 504–532.

Cort, W. W., McMullen, D. B., Olivier, L. and Brackett, Sterling 1940 Studies on schistosome dermatitis. VII. Seasonal incidence of *Cercaria stagnicolae* Talbot, 1936, in relation to the life cycle of its snail host, *Stagnicola emarginata angulata* (Sowerby). Am. J. Hyg. **32** (Sect. D): 33–69.

Cort, W. W. and Olivier, Louis 1941 Early developmental stages of strigeid trematodes in the first intermediate host. J. Parasitol. **27**: 493–501.

——— and ——— 1943 The development of the larval stages of *Plagiorchis muris* Tanabe, 1922, in the first intermediate host. J. Parasitol. **29**: 81–99.

Faust, E. C. and Hoffman, W. A. 1934 Studies on Schistosomiasis mansoni in Puerto Rico. III. Biological studies. I. The extramammalian phases of the life cycle. Puerto Rico J. Pub. Health and Trop. Med. **10**: 1–97.

Faust, E. C. and Meleney, H. E. 1924 Studies on Schistosomiasis japonica. Am. J. Hyg., Monogr. Ser. **3**: 1–339.

McMullen, D. B. and Brackett, Sterling 1941 The distribution and control of schistosome dermatitis in Wisconsin and Michigan. Am. J. Trop. Med. **21**: 725–729.

Miyairi, K. and Suzuki, M. 1914 Der Zwischenwirt des *Schistosomum japonicum* Katsurada. Mitteil. Med. Fak. Kaiserl. Univ. Kyushu, **1**: 187–197. (Cited from Faust and Meleney, 1924.)

Price, Helen, F. 1931 Life history of *Schistosomatium douthitti* (Cort). Am. J. Hyg. **13**: 685–727.

TANG, C. D. 1938 Some remarks on the morphology of the miracidium and cercaria of *Schistosoma japonicum*. Chinese Med. J., Suppl. **2**: 423–432.
WALL, L. D. 1941 *Spirorchis parvus* (Stunkard), its life history and the development of its excretory system (Trematoda: Spirorchiidae). Tr. Am. Micr. Soc. **60**: 221–26.

THE MODIFICATION OF THE DIGESTIVE GLAND TUBULES IN THE SNAIL *STAGNICOLA* FOLLOWING PARASITIZATION

IVAN PRATT AND WILLIAM D. LINDQUIST
Department of Zoology, University of Idaho, Moscow

As early as 1915 Cort noticed that the livers of the snail, *Lymnaea reflexa*, infected with *Cercaria douthitti*, were filled with a tangled mass of elongate, cylindrical sporocysts.

Faust (1917) suggested that a mechanical pressure of the larval stages of certain trematodes tended to affect the glandular organs by inhibiting or increasing their function and cramping the tissues within unusual confines.

Agersborg (1924) stated that the effect of such a trematode infection could be divided into four phases. There was a distorted and disintegrated condition of the tissues. The tissues reacted to the invasion by the production of black granules which were discharged into the intracellular spaces. A very distorted condition existed in the liver with the epithelium nearly destroyed, producing the effect of squamous rather than the normal columnar epithelium. If the snail survived there was a return to the normal condition.

Sometimes infected snails were abnormally large. Wesenberg-Lund (1934) first pointed out this fact. He advanced the idea that size might not be a criterion of the age of the snail, but that the excessive growth was due to the presence of parasites. This increase in size he attributed to the fact that the snails might have ingested abnormally large quantities of food in order to satisfy the demands of the parasites as well as themselves.

Rothschild has studied the phenomenon of gigantism in infected snails. In 1936 she recorded the relationship of large size and parasitism in the snail *Peringia ulvae*. In 1941 she determined by x-ray sterilization that the increase in size of the snails was not due to castration by the parasite. The abnormal size of the infected snails seemed to be a result of effects of the parasite upon the digestive gland of the snail.

Brackett (1940) found that snails brought into the laboratory often failed to emit cercariae until after they had been fed or recommenced after a starvation period.

Cort, Olivier, and McMullen (1941) stated that old infections of larval trematodes almost completely destroyed the digestive glands of the infected *Physa parkeri*. Their method of determining the destruction was not explained.

Pratt and Barton (1941) noted that the effect of a heavy infection was to reduce the apparent number of liver tubules and to limit them to the periphery of the liver area. The parasites were found in the central area of the liver or digestive gland and scattered between the liver tubules. No parasites were found in the centers of the liver tubules, the hermaphroditic duct, the ovotestis or the intestine.

The present work was a continuation of the work by Pratt and Barton. An effort was made to determine the effect of the infection upon the number and distribution of the liver tubules and upon their histology.

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MATERIALS AND METHODS

The snails studied were *Stagnicola emarginata angulata* (Sowerby). They were collected from Douglas Lake, Michigan. The cercariae from each snail were identified by Dr. Sterling Brackett while they were alive. The snail was then extracted from the shell and fixed in Bouin's or Gilson's fluid.

The fixed material was embedded in paraffin and serially sectioned 14 microns thick, stained in Semicon's carmine, dehydrated and mounted in damar.

The cercariae coming from the snails were identified as *Cercaria laruei* (Cort and Brooks, 1928), and *Diplostomum flexicaudum* (Cort and Brackett, 1937). All snails studied had been shedding only one kind of cercariae at the time that they were killed. A snail which was not shedding cercariae was studied for comparisons with the infected snails.

Each liver tubule that was large enough to have a lumen and each parasite that showed a distinct limiting membrane was counted in two snails, one infected with *Cercaria laruei* and the other that proved to be without parasites. A survey of these data indicated that counting the liver tubules and parasites in every tenth section gave a representative picture of the material. The remaining two snails studied were analyzed in this manner. The words digestive gland and liver are used synonymously in this paper.

DATA

The snail that was not parasitized (Snail A) was characterized by a digestive gland that tapered fairly smoothly at both ends. The twisted condition of the organ accounted for the fact that the posterior or spire end of the gland terminated abruptly as it did in two of the other snails. The maximum breadth of the gland occurred between the 100th and 200th sections which constituted the second fourth of the gland from the anterior end. The maximum number of tubules in any one cross section was 102. The total length of the digestive gland was about 5.0 mm, but this specimen was fixed in Gilson's fluid while the other ones were fixed in Bouin's. The Gilson's fluid caused the material to shrink more than did Bouin's.

Snail B was infected with the trematode *Diplostomum flexicaudum*. In the anterior end of the digestive gland there was sufficient room so that the parasites did not crowd the liver tubules. At about the hundredth section, which was approximately 1.4 mm back, the parasites became numerous. Fifty and more parasites were counted in each cross section. The liver tubules in this area differed from those in the corresponding area in the uninfected snail in that the number of them was reduced. Instead of this being the region where the tubules were the most numerous, as it was in the uninfected snail, the maximum number of tubules was found much farther posterior in the gland. They reached the greatest number at the beginning of the last fourth of the gland. As compared to the other snails studied this was the lightest infection. In no cross section were there as many as a hundred parasites. The number of parasites decreased toward the posterior or spire end of the digestive gland, but 3 were found in the last section.

Snail C was infected with *Cercaria laruei*. This was the heaviest infection of the three studied. The gland from the 50th to the 250th section contained large numbers of parasites, as many as 224 in one section. This part of the gland was the one in which the maximum number of tubules was found in the uninfected snail.

The effect of the parasites was apparently to spread out the liver tubules. At the upper end of the gland there were no parasites in Snail C. The gland was approximately 6.7 mm in length and constituted 480 cross sections.

TABLE 1.—*Serial sections of the digestive glands of* Stagnicola emarginata angulata. *Comparison of number of digestive gland tubules and parasites present in every 10th section. Snail A not infected. Snail B infected with* Diplostomum flexicaudum. *Snails C and D infected with* Cercaria laruei

Serial section	Snail A	Snail B		Snail C		Snail D	
	tubules	Tubules	Parasites	Tubules	Parasites	Tubules	Parasites
1	10	2	37	17	19	3	14
10	26	4	28	14	40	8	12
20	25	14	24	21	58	14	19
30	27	24	16	23	74	26	19
40	29	31	17	28	78	35	27
50	29	32	22	26	96	31	39
60	41	33	17	26	111	40	41
70	64	38	24	38	64	44	48
80	68	49	33	29	95	52	56
90	50	52	41	32	79	49	57
100	77	38	50	23	118	24	56
110	70	30	52	29	90	34	79
120	102	33	60	49	224	39	107
130	84	19	65	62	127	24	88
140	82	28	84	74	176	24	91
150	79	29	91	60	100	25	85
160	84	37	77	50	44	37	116
170	77	60	92	96	114	37	109
180	87	51	83	83	167	51	112
190	77	70	86	95	166	68	86
200	65	47	70	58	164	65	92
210	48	57	65	60	99	69	86
220	65	98	64	68	92	66	102
230	62	82	75	72	87	62	110
240	45	74	85	63	80	35	139
250	52	87	83	60	59	36	143
260	50	63	87	64	61	52	156
270	31	68	88	55	69	25	154
280	21	62	78	60	54	39	120
290	19	77	84	59	65	30	132
300	24	81	98	66	65	42	123
310	30	90	81	70	48	49	110
320	14	87	90	69	31	45	85
330	13	104	91	80	28	52	78
340	17	77	89	78	17	43	73
350	24	78	77	78	16	43	44
360	12	70	74	65	19	42	45
370	17	58	64	66	13	43	33
380		42	57	63	17	34	50
390		45	33	63	8	31	59
400		36	16	57	10	38	63
410		36	3	75	11	39	61
420				52	11	32	35
430				59	6	30	35
440				46	7	28	31
450				43	1	29	22
460				13	0	34	18
470				17	0	38	23
480				16	0	19	22
490						16	18
500						21	30
510						27	28
520						18	39
530						18	37
540						16	26
550						5	32
560						11	9
570						5	3
578						0	2

Snail D, which was also infected with *Cercaria laruei,* showed the suppression of liver tubules in the part of the digestive gland where the parasites were most numerous in a distinct pattern. From section 100 to section 180 the parasites were numerous. In that part of the gland the liver tubules numbered in the twenties and thirties, although the number of tubules below the concentration of parasites was around 50 per microscopic section. The number of parasites fell off in the region of sections

190 to 210 and in the same region the number of tubules was in the sixties. Again at section 220 the parasites increased in number and were numerous back to section 310. Again the number of liver tubules dropped back into the twenties and thirties. The suppressing effect of the heavy parasitization was evident up to about 20 sections (0.28 mm) from the margin of the mass of parasites at both ends of the parasitic mass. The parasites and liver tubules gradually tapered off toward the spire end of the gland and ended almost together. The total length of this gland was about 8.1 mm.

The gland tubules of Snails A and D and the parasites in Snail D were counted for each section of the entire gland. A tubule appeared to extend for about ten sections. Therefore each tubule was counted a number of times. However the same procedure was followed in both snails. Snail A, which was not parasitized, contained 20,417 sections of liver tubules; Snail D, which was fairly heavily parasitized, contained 20,105 sections of liver tubules. The difference in number of tubules between the uninfected and the parasitized snail was insignificant.

The effect of the parasites upon the histology of the liver tubules was similar to that found by Agersborg (1924). In the immediate vicinity of the parasites the liver tubules were reduced from a columnar epithelium to a cuboidal or flattened type. In adjacent areas the unaffected columnar epithelium was characterized by a conspicuous layer at the lumen end of the cells. This layer was absent in the cells of tubules immediately adjacent to parasites. These latter cells did not stain as sharply as did the unaffected cells. Droplets within the columnar cells showed up very clearly in the preparations as empty spaces. These clear areas were also present in the flattened cells found adjacent to parasites. The delicate connective tissue network was much disrupted by the parasites as noted by Pratt and Barton (1941).

DISCUSSION

When the entire number of cross sections of Snail A were counted there was a fairly regular increase and decrease in number of liver tubules. Examination of the microscopic slides suggested that the gland branched something like a fir tree. The maximum number of tubules corresponded to the branches of the tree and the minimum between two maxima corresponded to the spaces between successive branches from the main tree stem. There was a main opening into the intestine just above the lower end of the gland and a main duct from which the secondary branches sprung.

Cort, Olivier and McMullen (1941) found that the livers of *Physa parkeri* were almost completely destroyed by the larval trematode infections. To the contrary, this present work did not disclose convincing evidence of destruction or decrease in amount of liver tissue.

The layer on the lumen end of the cells of unaffected columnar epithelium certainly resembled a secretory layer as seen in vertebrate glands. The tubules in the region of parasites showed an histological change toward a tissue that probably was not secreting, characterized by flattened cells making up the wall of the tubules. It is possible that the presence of the parasites caused the tubules to go through a secretory cycle and become exhausted. There might be a relationship between these histological data and the observations of Wesenberg-Lund (1934) and Rothschild

(1936, 1938, and 1941) that parasitized snails are larger* than are the snails of the same age and population that are free of larval trematodes.

Although the number of liver tubules was fewer in the parts of the gland where the parasites were most numerous, the evidence suggests that the tubules were merely displaced posteriorward. The data indicate that the infection had to be quite heavy in order to produce the regional reduction in number of liver tubules.

In preparing the digestive glands of snails for the study, several sets of slides were made of the remainder of the tissues of the snails. This technique was difficult because certain tissues became very hard and brittle and fell away from the paraffin ribbon. However, in those specimens where this fault did not occur, it was possible to get some idea of the relationship of the parasites to the other organs of the body of the snail. Although the parasites were not numerous, there were stages other than cercariae in the body cavity lying free among the organs.

SUMMARY

1. Four snails identified as *Stagnicola emarginata angulata* were fixed, serially sectioned, stained, mounted in damar and studied to ascertain the effect of larval trematodes upon the digestive gland.

2. Snail A was found to be free of trematode infection and was studied in detail to discover the normal distribution of liver tubules in the digestive gland of this species of snail, and the histological characteristics of the cells making up the tubules. The liver was formed something like a fir tree with the greatest bulk occurring in the second fourth from the anterior end of the gland. The cells making up the ultimate branchings of the gland constituted a columnar epithelium. A layer in these columnar cells adjacent to the lumen of the liver tubule appeared to be secretory in nature. Droplets, in addition to the layer, were found throughout the length of the cells.

3. Snail B was infected with *Diplostomum flexicaudum*. This infection was fairly light with never as many as a hundred parasites in a single cross section. The effect of the infection was to cause the maximum number of liver tubules to be found farther posteriorward than in the uninfected snail.

4. Snail C infected with *Cercaria laruei* harbored the heaviest infection of those studied. The greatest number of parasites was in the area from about one-tenth to one-half of the way back, and the liver had the most tubules about two-thirds of the way toward the spire end where the number of parasites was about one-sixth as great as farther forward.

5. In Snail D, which was infected with *Cercaria laruei*, the parasites were grouped into two main masses and the liver tubules were suppressed in both those regions, although the effect of the crowding did not show in the number of liver tubules for about 0.28 mm within the mass of parasites.

6. The total number of sections of liver tubules was counted in Snails A and D. The uninfected snail had 20,417 and the infected snail 20,105 sections of liver tubules. There had thus been no destruction of liver tubules in Snail D as judged by the tubule section counts.

* Rothschild (1938) was misquoted in the paper by Pratt and Barton (1941). In paragraph 4 of the latter the text should have read ". . . *Peringia ulvae* when parasitized by trematodes were *larger* than other individuals of the same age that were not parasitized."

7. The tubules in the vicinity of the parasites showed a modification of structure from cells of columnar type to cuboidal or squamous type. The unaffected cells not adjacent to parasites were like those in the non-parasitized snail.

8. Although there was no secretory layer on the affected cells, there were droplets within the flattened cells.

9. A possible relationship between the modifications of the cells adjacent to the parasites and the gigantism among parasitized snails was suggested.

References

Agersborg, H. P. K. 1924 Studies on the effect of parasitism upon the tissues. I. With special reference to certain gastropod molluscs. Quart. Jour. Micr. Sc. **68**: 361–401.

Brackett, Sterling 1940 Studies on schistosome dermatitis. VI. Notes on the behavior of schistosome cercariae. Am. J. Hyg. **31**: 64–73.

Cort, W. W. 1915 Some North American larval trematodes. Illinois Biol. Monogr. 1: 453–532.

Cort, W. W. and Brackett, Sterling 1937 Two new species of strigeid cercariae from the Douglas Lake Region, Michigan. J. Parasitol. **23**: 265–280.

Cort, W. W. and Brooks, S. T. 1928 Studies on the holostome cercariae from Douglas Lake, Michigan. Tr. Am. Micr. Soc. **47**: 179–221.

Cort, W. W., Olivier, L. and McMullen, D. B. 1941 Larval trematode injection in juveniles and adults of *Physa parkeri* Currier. J. Parasitol. **27**: 123–141.

Faust, E. C. 1917 Life history studies on Montana trematodes. Illinois Biol. Monogr. **4**: 1–120.

Pratt, Ivan and Barton, G. D. 1941 The effects of four species of larval trematodes upon the liver and ovotestis of the snail. *Stagnicola emarginata angulata* (Sowerby). J. Parasitol. **27**: 283–288.

Rothschild, Miriam 1936 Gigantism and variation in *Perinoia ulvae* Pennant 1777 caused by infection with larval trematodes. J. Marine Biol. Assn. **20**: 537–546.

———1938 Further observations on the effect of trematode parasites on *Perinoia ulvae* (Pennant) 1777. Novit. Zool. **41**: 84–102.

———1941 The effect of trematode parasites on the growth of *Littorina neritoides* (L.). J. Marine Biol. Assoc. **25**: 69–80.

Wesenberg-Lund, C. 1934 Contributions to the development of the Trematoda Digenea. Part II. The biology of the fresh-water cercariae in Danish fresh-waters. Danske Vidensk. Selsk. Skr. Naturv. og Math. Afd. (Mem. Acad. Roy. Sc. et Lettr. Danemark. Copenhague, Sect. Sc.) **5**: 1–223.

TWO NEW LARGE-TAILED CERCARIAE (PSILOSTOMIDAE) FROM NORTHERN MICHIGAN*

LESLIE R. HEDRICK

In the summer of 1941 the author studied some of the larval trematodes of the smaller snails of the Douglas Lake region in Michigan. The bottom vegetation and ooze where the snails abide was scooped up with a large galvanized pan provided with a brass screen bottom. This material containing the snails was placed in a wash basin and the snails were separated from the lighter debris by a series of decantations. This was usually done in the field. Rock pebbles were often with the snails after the other material had been removed. At the laboratory the snails and rocks were placed in a large, glass, flat-bottom container and swirled with water so that the snails and rocks were in the center of the dish. After a few hours, nearly all the snails had wandered toward the periphery of the dish free from the rocks. Then the snails were separated into genera and species. Ten to fifteen snails of a kind were placed in a Syracuse watch glass with enough water to cover the animals.

The next day these dishes were examined for the emergence of cercariae. Approximately 10,000 *Amnicola limosa* Say were collected, some of which gave emergence to three different large-tailed forms which probably belong to the psilostome group. The names *Cercaria ameeli* n. sp. and *Cercaria limosae* n. sp. are proposed for two of these. The other form was found only once and not enough data were recorded for an adequate description. Descriptions of the cercariae and rediae will follow. All the cercaria measurements were made from specimens killed in 4% formalin, heated to 50° C. The rediae were measured from living material.

Cercaria ameeli n. sp.

(Figs. 1–3)

Specific diagnosis: Psilostome cercaria (Fig. 1). Cuticular spines limited to anterior region of body, extending posteriorly only to level of middle of anterior sucker; remainder of body wall spineless but with thick granular cuticle. Oral sucker terminal and unspined, very muscular; approximately same size as acetabulum. Prepharynx rather long; pharynx pyriform and robust, more than one-half length of oral sucker. Esophagus clear, wider than pharynx. Intestinal ceca clear, branch just anterior to acetabulum and extend more than half way around acetabulum. Acetabulum near posterior end of body, large, concave, and protrudes extensively from ventral body surface. Excretory bladder two chambered; anterior, rectangular chamber in body empties into posterior, triangular chamber in tail by narrow tube. Major collecting ducts arise from anterio-median margin of anterior chamber and quickly diverge posterior to acetabulum to extend in lateral field to near anterior end of body. Flame cell pattern, five pairs on either side; flame cells rather large. No penetration glands found but two "glandular" areas present in anterior end of body on either side of oral sucker and prepharynx. Posterior to these areas, body contains many polygonal cystogenous bodies with fine rod-like striations.

Tail is more than twice length of body and wider than body (Fig. 2); flattened and ribbon shaped with smooth margins when relaxed. Color of tail yellow-amber. Seven to nine pairs of clusters of large amber granules in two lateral areas of tail. These large granules not definitely attached to any structure but have limited range of flow in lymph of tail. Small yellow uniformly distributed granules diffusely scattered throughout tail. Triangular chamber of excretory bladder in anterior end of tail terminates in short blind tube.

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Measurements of thirty specimens; mean in microns with its probable error; extremes in parenthesis. Body length 213 ± 2.5 (185–281); body width 99 ± 1 (77–122); tail length 447 ± 7.5 (339–585); tail width 121 ± 2.5 (90–145); oral sucker diameter 39 ± 2 (35–42); acetabulum diameter 40 ± 2 (35–48); distance of acetabulum to posterior end of body 22 ± 0.5 (14–28); prepharynx length 23 ± 1 (14–34); pharynx length 26 ± 0.5 (17–31); pharynx width 14.

Host: Amnicola limosa Say.

Locality: Douglas Lake, Cheboygan County, Michigan.

REDIAE OF *C. ameeli*

The redia of *C. ameeli* has a well-developed sucker and a very prominent pharynx. The intestine extends to the posterior end of the body and is a rich chestnut brown in color. The birth pore is just posterior to the pharynx (Fig. 4). The length of three rediae ranged from 0.9 to 1.2 mm. The width ranged from 0.22 to 0.26 mm. The length of the redial pharynx is 65 μ; the width of the pharynx is 52 μ. The rediae were rather active. The tails of very young cercariae within the rediae are distinctly granular, but the granules are colorless and refractory. In more developed immature cercariae within the rediae, the granules are colored and much more numerous than in mature cercariae. Colorless granules surround the cercariae within the body of the redia.

Approximately fifteen *Amnicola limosa* of the 10,000 collected produced *Cercaria ameeli*. The daily output is thirty to fifty cercariae. The cercariae swim on their sides with the tail having the appearance of an undulating ribbon. The tail usually pushes but it may also pull the body. When the cercariae rest on the bottom of the dish, the tail is straight. Cercariae which are relaxed and suspended in the water tend to come to the surface. The tails of relaxed cercariae in a vial of water are straight and in a vertical position. These tails apparently support the smaller bent cercarial body which hangs below (Fig. 3).

Cercaria limosae n. sp.

(Figs. 5, 7)

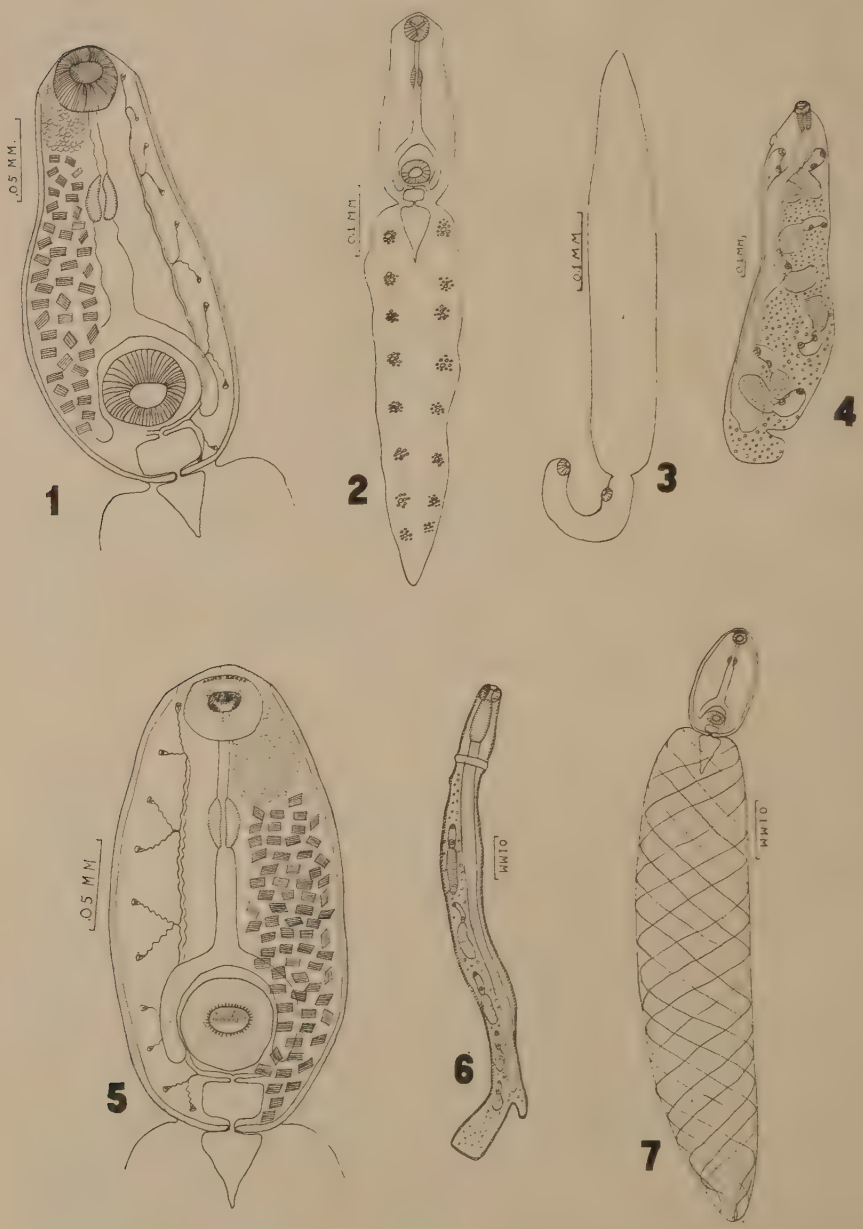
Specific diagnosis: Psilostome cercaria (Fig. 5). Cuticular spines limited to anterior region of body, extending posteriorly only to level of middle of anterior sucker; remainder of body wall spineless but with thick granular cuticle. Oral sucker terminal, very muscular with ten rather broad spines on anterior border; approximately same size as acetabulum. Prepharynx rather long; pharynx pyriform and robust, more than one-half length of oral sucker. Esophagus clear, wider than pharynx and extending posteriorly nearly to acetabulum, where it branches to form two clear ceca which extend more than half way around acetabulum. Acetabulum near posterior end of body; large, protrusible; distal margin with thirty-six rather broad spines. Excretory bladder two chambered; anterior, rectangular chamber in body empties into posterior, triangular chamber in tail by narrow tube. Major collecting ducts arise from anterio-median margin of anterior chamber and quickly diverge posterior to acetabulum to extend in lateral field to near anterior end of body. Flame cell pattern five pairs on either side; flame cells rather large. No penetration glands found but two "glandular" areas present in anterior end of body on either side of oral sucker and prepharynx. Posterior to these areas, body has many polygonal cystogenous bodies with fine rod-like striations.

Tail rufous brown in color and appears reticulated with interlacing lymph canals (Fig. 7). Pigment in small granules uniformly distributed. Tail four to five times as long as body and nearly twice as wide. Triangular excretory chamber in anterior end of tail ends in a blind narrow sac. Cercaria swims on its side and tail in swimming motion appears to be undulating ribbon.

Measurements of fifteen specimens; mean in microns with its probable error; extremes in parenthesis: body length 228 ± 1.5 (195–270); body width 107 ± 1.5 (93–124); tail length 833 ±34.5 (570–1050); tail width 202 ± 6.5 (155–248); oral sucker diameter 42 ± 1 (37–56); acetabulum diameter 44 ± 0.6 (40–55); distance of acetabulum to posterior end of body 21 ± 0.7 (16–28); prepharynx length 23 ± 1 (18.5–26); pharynx length 23 ± 0.6 (19–31).

Host: Amnicola limosa Say.

Locality: Douglas Lake, Cheboygan County, Michigan.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During the course of the summer, five *Amnicola limosa* were found to be infected with *Cercaria limosae*. Each snail releases approximately ten cercariae per day. However, during the warm weather the snails only lived in the laboratory two or three days and seldom did I have over one infected snail at any one time, so I was unable to carry out any extensive feeding experiments. When the cercariae relax, the tail is straight and flat. This species was not observed in a vertical container, so I am unable to report whether these forms hang in the water with the tail upward and the body downward.

REDIAE OF *C. limosae*

The redia has a collar, a well-developed sucker and a large pharynx (Fig. 6). Birth pore posterior to collar. Intestine, which extends to near the posterior end, filled with chestnut brown granules. Epithelioid layer just under the body covering, amber in color. Caudal appendages near posterior end. Length of mature rediae 1.5 to 2.7 mm. In the developing cercariae, the posterior apex of the tail portion of the bladder extends in a median line two-thirds the length of the tail. Here it divides into two branches, each of which opens to the exterior on the sides of the tail. In the fully-developed cercariae, both before and after emergence, this posterior extension from the apex of the tail portion of the bladder is absent.

INFECTION EXPERIMENTS

Several series of experiments to determine the second intermediate host for *C. ameeli* were negative. Small fish or tadpoles were placed in a finger bowl containing a known number of cercariae (usually 40 to 100). In several instances of direct observation with a binocular dissecting microscope, the cercarial body and tail were drawn into the mouth or external nares with the respiratory current. The suspected host was left with the cercariae for several hours or overnight. Then the number of remaining cercarial bodies and cercarial tails was determined in order to ascertain the number of cercarial bodies in the experimental host.

In ten such experiments with young *Perca flavescens,* no metacercariae were found either after an interval of one or two days, or after a three-week interval. In all of these perch, 25 to 50 cercarial bodies were determined to have entered. Likewise, results were negative when young bullheads, guppies and tadpoles were used. In experiments with *C. limosae,* the methods used were identical with those described for *C. ameeli* except that I worked with smaller numbers of cercariae, namely ten to twenty. The results were also negative.

DISCUSSION

A comparison of the two forms described above with the cercariae of the four life histories described for the psilostome group reveals general relationships, but

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FIG. 1. Body of *Cercaria ameeli,* anterior glandular area and cystogenous glands show on one side; flame cell arrangement on the other side.

FIG. 2. Outline of entire *Cercaria ameeli,* showing location of large granules in tail.

FIG. 3. *C. ameeli* in natural resting position in water.

FIG. 4. Redia containing young *C. ameeli.*

FIG. 5. Body of *C. limosae* with flame cells shown on one side and glandular area and cystogenous glands on the other side.

FIG. 6. Redia containing young *C. limosae.*

FIG. 7. Outline of entire *Cercaria limosae* showing reticular character of tail due to interlacing lymph canals.

also some differences. Life histories for four Psilostomes have been described—*Psilotrema spiculigerum* (Mühling) by Mathias (1925), *Sphaeridiotrema globulus* (Rud.) by Szidat (1937), *Psilostomum ondatrae* Price by Beaver (1939) and *Psilostomum reflexae* (Cort) by Feldman (1941). In the cercariae of all of these forms, there is a definite excretory siphon which recurves backward near the anterior sucker. In the forms just described, no siphon was found and the collecting ducts did not recurve backward and neither did they contain granules.

The flame cell patterns for the cercariae of *Psilotrema spiculigerum* and *Sphaeridiotrema globulus* were not described. In the cercariae of *Psilostomum ondatrae*, as reported by Beaver, the flame cells were in eight groups of three on each side of the body. Feldman described the flame cell arrangement for *Psilostomum reflexae* as forty-two pairs. The flame cell pattern for both *C. ameeli* and *C. limosae* is five groups of two on either side. For general appearance and swimming action, the cercariae I have described compare very well with the cercariae of *S. globulus* as described by Dú Bois for *C. helvetica* XVII, 1928 and Szidat, 1937.

The arrangement of the excretory bladder and the shape and general structure of the tail of my forms are somewhat similar to those described for the "amphistome cercaria" of Peterson, 1931, and *Cercaria obscura* of Wesenberg-Lund, 1934.

SUMMARY

Two new large-tailed psilostome cercariae, *C. ameeli* n. sp. and *C. limosae* n. sp., and their respective rediae are described from *Amnicola limosa* Say of the Douglas Lake Region.

Infection experiments upon young perch, young bullheads, guppies and tadpoles to determine the second intermediate hosts were negative.

References

Beaver, P. 1939 The morphology and life history of *Psilostomum ondatrae* Price, 1931 (Trematoda: Psilostomidae). J. Parasitol. **25**: 383–393.

Dú Bois, G. 1928 Les cercaires de la région de Neuchatel. Bull. Soc. Neuchat. Sc. Nat. **53**: 3–177.

Feldman, S. 1941 Studies on the morphology and biology of a psilostome fluke. J. Parasitol. **27**: 525–533.

Mathias, P. 1925 Recherches expérimentales sur le cycle évolutif de quelques trématodes. Bull. Biol. France et Belgique **59**: 1–123.

Petersen, H. 1931 Cercarien der Niederelbe. Zool. Anz. **97**: 13–27.

Szidat, L. 1937 Über die Entwicklungsgeschichte von *Sphaeridiotrema globulus* Rud. 1814 und die Stellung der Psilostomomidae Odhner in natürlichen System I. Z. Parasitenk. **9**: 529–543.

Wesenberg-Lund, C. 1934 Contributions to the development of the Trematoda Digenea. Part II. The biology of the freshwater cercariae in Danish freshwater. Danske Vidensk. Selsk. Skr. Naturv. og Math. Afd. (Mem. Acad. Roy. Sc. et Lett. Danemark, Copenhagen, Sec. Sc.) **5**: 1–223.

THE OCCURRENCE OF BLOOD PARASITES IN BIRDS FROM SOUTHWESTERN UNITED STATES[1]

SHERWIN F. WOOD AND CARLTON M. HERMAN

Department of Life Sciences, Los Angeles City College and
Los Angeles Wildlife Disease Research Station

A preliminary report on blood parasites of birds in the Southwest was published in 1937 by Wood and Wood. The present contribution includes the bird material of the earlier paper and therefore supplements that report. The slides that constitute the basis of this study were prepared at various times from November, 1935 through August, 1939. Most of the blood smears were made in 1937 and 1938. The number of slides prepared by each cooperator is as follows: William G. Webb (1), Walter Nichols (22), Professor Loye H. Miller (45), Carlos Stannard (58), P. J. Van Huizen (70), Josephine Michener (588), and F. D. and S. F. Wood (741).

MATERIALS AND METHODS

Most of the bird blood smears used in this study were made from banded birds by piercing the brachial vein on the under side of the left wing with a surgical needle. Others were made from gunshot wounds or internal organs. Therefore, barring natural destruction, most of these birds are still in the field. Band numbers of infected birds will be furnished other investigators on request to the senior author. The smears were air dried and stained in the laboratory with Jenner-Giemsa stain from one to many days after preparation.

All stained blood smears were examined for at least 20 minutes: 1 to 5 minutes with low power (6× oculars and 16 mm stirrup objective) and 15 minutes with the oil immersion lens (6× oculars). A count of the number of blood cells on a smear 1 cell thick during a 15 minute search with oil reveals at least 220,000 blood cells inspected. The actual sample count made on a negative slide involved the survey of 996 oil immersion fields in the 15 minute period with 225 cells per field (average of 10 fields) for a total of 224,100 cells. Therefore, for every bird reported here, approximately this number of cells was examined on at least one slide.

OBSERVATIONS

Examination of blood smears from 1,525 birds revealed 357 or 23.4 per cent infected with *Trypanosoma, Haemoproteus, Plasmodium, Leucocytozoon, Hepato-*

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Success in finding parasites readily in smears is in some measure dependent upon the quality of the blood smear received. Special commendation in this regard is hereby expressed to Josephine Michener, P. J. Van Huizen and Loye H. Miller. The writers are indebted to Mr. George Willett of the Los Angeles Museum and Dr. Alden H. Miller of the University of California for advice on ornithological problems and to Dr. Fae D. Wood for her skillful execution of the drawings.

TABLE 1.—*The relative incidence of blood parasites in southwestern birds*

Common name	Scientific name	Number examined	Number infected	T.	HAEM.	P.	L.	HEP.	ILP.	M.	Infected localities
Anthony green heron	*Butorides virescens anthonyi*	1	1	1	...	1	1	..	..	..	Pasadena, Calif.
Cackling goose	*Branta canadensis minima*	5	1	..	...	..	1	..	..	..	Willows, Calif.
White fronted goose	*Anser a. albifrons*	5	1	..	...	..	1	..	..	..	Willows, Calif.
Common mallard	*Anas p. platyrhynchos*	15	2	..	1	..	2	..	..	1	Willows, Calif.
American pintail	*Dafila acuta tzitzihoa*	24	9	2	2	..	7	..	..	..	Willows, Calif.
Redhead	*Nyroca americana*	17	2	..	...	..	2	..	..	..	Willows, Calif.
Red breasted merganser	*Mergus serrator*	1	1	..	...	..	1	..	..	1	Pt. Mugu, Calif.
Sharp-shinned hawk	*Accipiter velox*	6	4	..	...	..	4	..	..	..	Pasadena, Calif.
Valley quail	*Lophortyx californica vallicola*	12	6	..	6	..	...	..	..	..	Eagle Rock (1), and Pasadena (5), Calif.
San Quintín quail	*Lophortyx californica plumbea*	33	14	..	14	..	...	..	..	..	Los Serranos Game Farm, Chino, Calif.
Gambel quail	*Lophortyx gambelii*	24	13	..	13	..	...	..	..	..	Phoenix, Ariz.
Mountain quail	*Oreortyx picta*	1	1	..	1	..	...	..	..	..	Mt. Piños, Calif.
Band-tailed pigeon	*Columba fasciata*	2	2	..	1	..	1	..	..	..	Peña Blanca Spring, Ariz., and Pasadena, Calif.
Western mourning dove	*Zenaidura macroura marginella*	27	26	..	26	..	4	..	..	..	Pasadena (4), Calif., and Phoenix (22), Ariz.
Chinese spotted dove	*Spilopelia chinensis*	25	1	..	...	..	1	..	..	..	Pasadena, Calif.
Western white-winged dove	*Melopelia asiatica mearnsi*	12	4	1	4	1	...	..	..	..	Phoenix, Ariz.
Spotted screech owl	*Otus trichopsis*	1	1	..	1	..	...	..	..	..	Peña Blanca Spring, Ariz.
California spotted owl	*Strix o. occidentalis*	1	1	1	1	..	1	..	..	1	Liebre Mts., Calif.
Eastern flicker	*Colaptes auratus*	1	1	1	...	..	...	..	..	..	Pasadena, Calif.
Western flycatcher	*Empidonax d. difficilis*	3	1	1	...	..	...	..	..	..	Pasadena, Calif.
California jay	*Aphelocoma c. californica*	29	10	..	...	3	9	..	..	..	Pasadena (9), and Los Angeles (1), Calif.
San Diego titmouse	*Baeolophus inornatus transpositus*	3	2	..	...	..	...	2	..	..	Pasadena, Calif.
Pallid wren-tit	*Chamaea fasciata henshawi*	1	1	..	...	..	...	1	..	..	Pasadena, Calif.
Rock wren	*Salpinctes obsoletus*	2	1*	..	...	..	...	..	..	..	San Nicolas Island, Calif.
Western mockingbird	*Mimus polyglottos leucopterus*	61	10	..	...	4	4	..	3	..	Pasadena (9), and San Fernando (1), Calif.
California thrasher	*Toxostoma r. redivivum*	14	2	..	...	..	1	1	..	..	Pasadena, Calif.
Western robin	*Turdus migratorius propinquus*	1	1	..	...	..	1	..	..	1	Shaver's Well, Calif.
Hermit thrush	*Hylocichla guttata*	3	1	..	...	..	1	..	..	..	Pasadena, Calif.
Russet-backed thrush	*Hylocichla u. ustulata*	3	1	1	1	..	1	..	..	1	Berkeley, Calif.
Orange crowned warbler	*Vermivora celata*	5	1	..	...	..	1	..	..	..	Pasadena, Calif.
Calaveras warbler	*Vermivora ruficapilla ridgwayi*	2	1	..	...	..	1	..	..	..	Pasadena, Calif.
Olive warbler	*Peucedramus olivaceus*	2	1	..	...	..	1	..	..	..	Chiricahua Mts., Ariz.
Yellow warbler	*Dendroica aestiva*	19	4	1	...	..	3	..	..	..	Pasadena, Calif.
Audubon warbler	*Dendroica auduboni*	46	14	3	2	..	8	1	..	..	Pasadena, Calif.
Black throated gray warbler	*Dendroica nigrescens*	1	1	..	1	..	...	..	..	..	Pasadena, Calif.
Macgillivray warbler	*Oporornis tolmiei*	1	1	1	...	..	...	..	..	..	Borrego, Calif.
Chat	*Icteria virens*	1	1	1	...	..	...	..	..	..	Pasadena, Calif.
Pileolated warbler	*Wilsonia pusilla*	9	2	1	...	..	...	..	..	1	Pasadena, Calif.
English sparrow	*Passer domesticus*	41	3	..	...	2	...	..	1	..	Pasadena, Calif.
Hooded oriole	*Icterus cucullatus*	18	15	4	11	2	...	..	...	2	Pasadena, Calif.
Bullock oriole	*Icterus bullockii*	4	4	3	4	..	1	..	..	..	Pasadena, Calif.
Brewer blackbird	*Euphagus cyanocephalus*	76	6	1	...	3	3	..	..	..	Pasadena, Calif.
Dwarf cowbird	*Molothrus ater obscurus*	55	7	2	...	2	3	..	1	..	Pasadena, Calif.
Western tanager	*Piranga ludoviciana*	16	12	4	9	..	2	..	..	..	Pasadena, Calif.
Black-headed grosbeak	*Hedymeles melanocephalus*	46	34	5	24	..	18	1	..	1	Pasadena, Calif.
Lazuli bunting	*Passerina amoena*	2	1	..	...	..	1	..	..	..	Pasadena, Calif.
Evening grosbeak	*Hesperiphona vespertina*	2	2	2	...	..	2	..	..	1	Chiricahua Mts., Ariz.
California purple finch	*Carpodacus purpureus californicus*	2	1	1	...	..	...	..	..	..	Pasadena, Calif.
Common house finch	*Carpodacus mexicanus frontalis*	530	69	..	13	51	5	2	2	..	Pasadena (46), and Los Angeles (23), Calif.
San Clemente house finch	*Carpodacus mexicanus clementis*	2	2	..	2	..	...	..	..	..	San Nicolas Island, Calif.
Willow goldfinch	*Spinus tristis salicamans*	33	5	4	...	..	1	..	..	..	Pasadena, Calif.
California towhee	*Pipilo fuscus crissalis*	29	1	..	...	1	...	..	..	..	Pasadena, Calif.
Gambel sparrow	*Zonotrichia leucophrys gambelii*	111	47	1	23	..	30	..	..	3	Pasadena (46), and Los Angeles (1), Calif.
San Diego song sparrow	*Melospiza melodia cooperi*	41	1	..	...	1	...	..	..	..	Pasadena, Calif.
Totals		1427	357	42	159	71	123	8	7	13	

Legend: T = *Trypanosoma*, Haem. = *Haemoproteus*, P. = *Plasmodium*, L. = *Leucocytozoon*, Hep. = *Hepatozoon*, ILP. = Intra-leucocytic Parasite, M. = Microfilaria.

* Infected with *Spirogregarina fusiformis*.

zoon, an intra-leucocytic organism, microfilarial worms, and a new parasite, *Spirogregarina fusiformis,* described below.

This survey included 112 species and subspecies of birds of which 54 species and subspecies or 48.2 per cent were infected. Credit for the extensive list of species is due entirely to the efforts of Josephine Michener and Loye H. Miller. Because of a shift of microscope equipment during this study, several light infections previously detected could not be verified even after extensive search of the slides. Therefore, the 23.4 per cent infection represents a minimum for the birds studied here. The data in Table 1 summarizes our findings. The birds are listed in the order in which they appear in the 1931 edition of the American Ornithologists' Union check-list.

Trypanosoma

Trypanosomes were found in 42 birds of 22 species or 11.8 per cent of the parasitized birds as shown in Table 1. These can be grouped somewhat as to type on the basis of morphology but no satisfactory species diagnosis can be made other than *Trypanosoma avium* Danilewsky until information is recorded on their life histories.

Haemoproteus

The genus *Haemoproteus* was observed in 159 birds of 21 species and subspecies or 44.5 per cent of the infected birds. *Haemoproteus lophortyx* O'Roke was found in the valley quail, San Quintín quail, Gambel quail and plumed quail. The great diversity of form characterizing this species creates the question of whether or not we are dealing with only one species in these birds. *Haemoproteus columbae* Kruse (= *maccallumi*) was found in the western mourning dove, western white-winged dove and the band-tailed pigeon. *Haemoproteus sacharovi* Novy and MacNeal was found in the western mourning dove and western white-winged dove.

The *Haemoproteus columbae* of the mourning dove showed gametocytes with irregular ends or regular, rounded ends. The latter structure was very similar to the gametocytes of *Haemoproteus* found in Audubon warbler, black-throated gray warbler, hooded oriole, Bullock oriole, western tanager, black-headed grosbeak, common house finch, San Clemente house finch, and Gambel sparrow.

A more robust type of *Haemoproteus* was seen in the spotted screech owl, the common mallard, and the American pintail.

Plasmodium

Parasites of the genus *Plasmodium* were found in 71 birds of 11 species or 19.9 per cent of the infected birds. The species encountered and their relative abundance in different kinds of birds is indicated in Table 2.

Leucocytozoon

Leucocytozoon were found in 123 birds of 33 species or 34.4 per cent of the infected birds.

Leucocytozoon simondi Mathis and Leger was found in 1 cackling goose, 1 white-fronted goose, 2 common mallards, 7 American pintails, 2 redheads, and 1 red-breasted merganser.

Leucocytozoon ziemanni (Laveran) was found in 1 California spotted owl. In 4 sharp-shinned hawks a parasite was found in which the cytoplasm of the host cell

was conspicuously short-tailed and whose host cell nucleus was less elongate than those parasitized by gametocytes of *Leucocytozoon simondi*.

Leucocytozoon in various stages of development characterized by the presence of a rounded form were seen in 1 Anthony green heron, 1 band-tailed pigeon, 4 western mourning doves, 1 Chinese spotted dove, 9 California jays, 4 western mockingbirds, 1 California thrasher, 1 western robin, 1 hermit thrush, 1 russet-backed thrush, 1 orange-crowned warbler, 1 Calaveras warbler, 1 olive warbler, 3 yellow warblers, 8 Audubon warblers, 1 Bullock oriole, 3 Brewer blackbirds, 3 dwarf cowbirds, 2 western tanagers, 18 black-headed grosbeaks, 1 lazuli bunting, 2 evening grosbeaks, 5 common house finches, 1 willow goldfinch, and 30 Gambel sparrows.

Hepatozoon

Hepatozoon was found in 8 birds of 6 species or 2.2 per cent of the infected birds. The organism occurred most frequently in lymphocytes and monocytes, tending to occupy most of the space available in the cytoplasm. Occasionally the position of the parasite resulted in a deep indentation on one side of the nucleus. The parasite

TABLE 2.—*Species of* Plasmodium *from southwestern birds*

Common Name	E	C	R	U	C & R
Anthony green heron	1	..	..	..	..
Western white-winged dove	..	..	..	1	..
California jay	..	..	1	2	..
Western mockingbird	..	1	2	1	..
English sparrow	..	..	1	1	..
Hooded oriole	..	..	2	..	..
Brewer blackbird	..	..	1	2	..
Dwarf cowbird	..	..	1	1	..
Common house finch	..	14	19	16	2
California towhee	..	..	1	..	..
San Diego song sparrow	..	..	1	..	..
Total	1	15	29	24	2

Legend: E = *elongatum*, C = *cathemerium*, R = *relictum* (*praecox*), U = Unidentified, and C & R = *cathemerium* and *relictum*.

had a characteristic elongate, spindle-shaped form as seen here in organisms from the San Diego titmouse, common house finch, and pallid wren-tit.

The nuclear chromatin was in strings, masses or granules dispersed band-like across the middle of the parasite's body, thus separating the two ends of the cell. In the San Diego titmouse the nuclear chromatin was definitely granular (Fig. 14), the granules merging into loose strands but sometimes forming irregular moniliform bands or in some specimens appearing as a band-like mass across the parasite (Fig. 13). In the pallid wren-tit most parasites showed a definitely banded nucleus (Fig. 18) although in some the chromatin appeared in coarse clumps.

The cytoplasm appeared finely granular or, as in the San Diego titmouse, definitely vacuolated giving the protoplasm an alveolar appearance. A few small pink-staining granules were seen in the cytoplasm having the same color as the nuclear material of the parasite.

In one end of the cell there was a circular vacuole (or less often two vacuoles = excretory ?) which had a very uniform, finely granular appearance (Figs. 13, 17). The substance of the vacuole seemed to shrink away from the vacuolar membrane creating a narrow lighter zone between the vacuolar substance and its membrane. There was usually only one vacuole per parasite (Fig. 11) and it was closely pressed to the side of the nucleus and separated from the end and sides of the cell membrane by alveolar protoplasm. In the San Diego titmouse (Figs. 13, 14), the vacuole was set off sharply from the surrounding cell cytoplasm.

The host cell nucleus was somewhat flattened against the parasite but usually not indented.

There were no purple-stained granules of variable size in the cytoplasm of *Hepatozoon* such as were seen in the intra-leucocytic parasites discussed below.

The single parasite from the California thrasher (Fig. 16) resembled *Hepatozoon* in internal structure but its shape was more like that of the intra-leucocytic parasites.

Two parasites found in an Audubon warbler (Figs. 11, 12) were in thrombocytes and the granular nucleus of the parasite tended to occupy a transverse, band-like zone near the middle of the cell.

Intra-Leucocytic Parasites

Similar organisms were figured by Hewitt (1940) from the common house finch. Parasites of this type were found in 7 birds of 4 species or 2.0 per cent of the parasitized birds studied here. Hewitt erroneously ascribed to this category a *Toxoplasma* parasite described by Wood and Wood (1937) from this species of bird. However, additional comparative material has revealed Fig. 12 of Wood and Wood (1937) to be a developmental stage of *Leucocytozoon* in the common house finch.

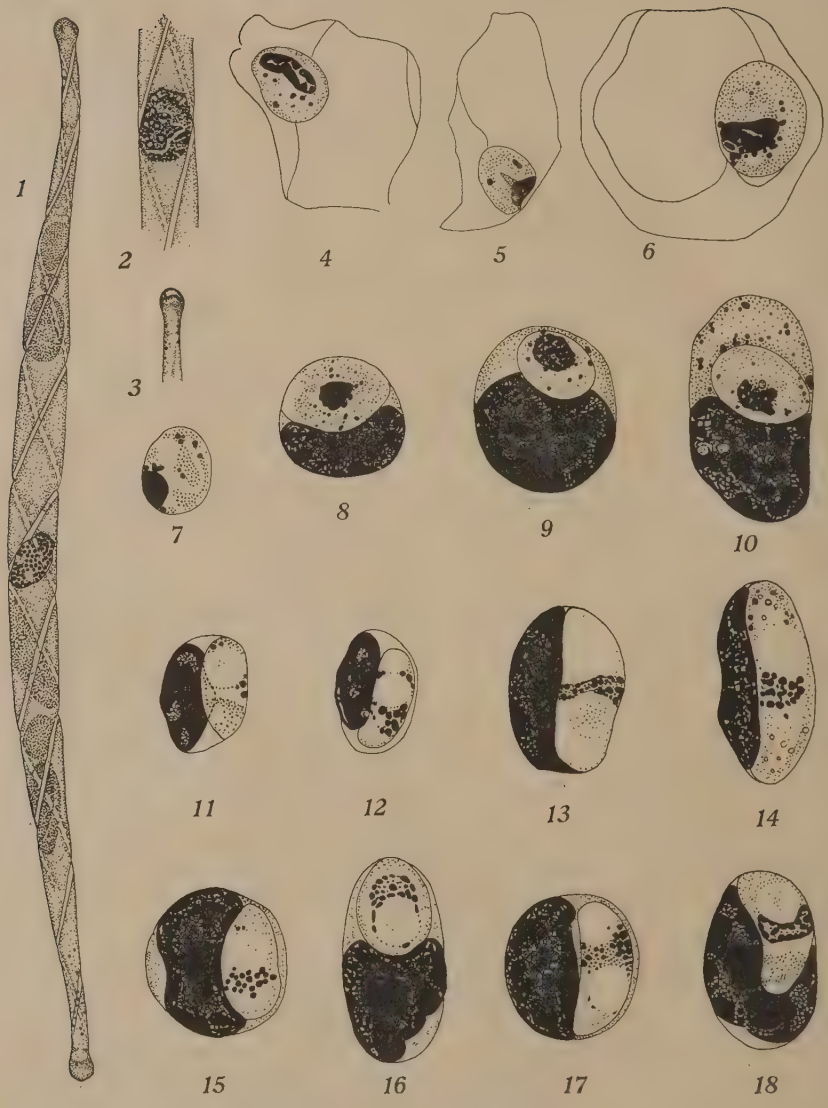
The intra-leucocytic parasite from the western mockingbird (Figs. 9, 10), English sparrow (Fig. 4), dwarf cowbird (Fig. 5), and common house finch (Figs. 6, 7, 8) was a small round or oval, intra-lymphocytic organism. All parasites appeared to be at about the same stage in development. There was a definitely pink-stained nuclear mass which occupies a central position. The pale-staining nucleus may be round, elongate or dispersed across the cell in a band-like form. It appeared to be very variable in shape but consisted of coarse chromatin blocks with intertwining narrow chromatin strands and narrow parachromatin spaces. In some cases, as when the intra-leucocytic parasite was turned on edge from being pushed to one side of the host cell (Figs. 5, 7), the nuclear structure appeared more compact and homogeneous resembling Fig. 456 (6–12) of *Hepatozoon* as seen in Wenyon (1926).

The cytoplasm of the parasite appeared uniformly vacuolated suggesting an alveolar pattern. In some organisms practically no structure was visible except a pale, fine granulation of variable density giving the cytoplasm a mottled appearance. In free parasites, frequently small clear vacuoles were seen. These were occasionally found in the intra-lymphocytic forms.

In the cytoplasm, there were few to many, fine or coarse, dark purple-staining granules which tended to clump about the nucleus and were sometimes superimposed upon it. Hewitt (1940) seemed to have confused these cytoplasmic granules with chromatin which always has a light pink tint in our preparations. However, some of the smaller granules do resemble the nuclear chromatin in color and their tendency to clump about the nucleus aids in their being confused with it. These cytoplasmic granules were large and distinctly purple in our preparations from the western mockingbird and common house finch in contrast with the pale pink of their nuclei.

The host cell nucleus showed slight to pronounced indentation where in contact with the parasite.

In this study, the chief differences noted between *Hepatozoon* and the intra-leucocytic parasites were: elongate spindle-shape vs. a round or oval form, presence or absence of purple-stained cytoplasmic granules, band-like nucleus vs. round or elongate nucleus, and presence or absence of a distinct large cytoplasmic vacuole.



Figs. 1–3, ×1500; Figs. 4–18, ×2588

FIG. 1. *Spirogregarina fusiformis* n. g., n. sp., from the San Nicolas rock wren showing typical structure.

FIG. 2. Nuclear area of another *Spirogregarina fusiformis* showing irregularly-shaped nucleus surrounded by coarse granular mass.

FIG. 3. One extremity of another *Spirogregarina fusiformis* showing bulbous end suggesting a flattened or indented surface.

FIG. 4. Intra-leucocytic parasite in a monocyte from an English sparrow. Crushed host cell nucleus (to the right) and cytoplasm outlined.

FIG. 5. Intra-leucocytic parasite in a medium-sized lymphocyte from a dwarf cowbird. Host cell nuclear outline is to the right.

Microfilaria

Microfilarial worms were found in 13 birds of 10 species or 3.6 per cent of the infected birds. The total number found on one blood smear and the approximate size of single specimens is as follows: common mallard, 9 unsheathed forms (104 $\mu \times 4 \mu$); red-breasted merganser, 5 unsheathed forms (296 $\mu \times 6 \mu$); California spotted owl, 247 sheathed forms (242 $\mu \times 8 \mu$) and 175 unsheathed forms (92 $\mu \times 5 \mu$); western robin, 1 sheathed (140 $\mu \times 4 \mu$); russet-backed thrush, 336 unsheathed (153 $\mu \times 4 \mu$); pileolated warbler, 8 sheathed (94 $\mu \times 4 \mu$); hooded oriole, 4 unsheathed forms (39 $\mu \times 3 \mu$) in one bird and 135 unsheathed forms (56 $\mu \times 4 \mu$) in another bird; black-headed grosbeak, 2 sheathed forms (118 $\mu \times 3 \mu$); evening grosbeak, 8 unsheathed forms (69 $\mu \times 4 \mu$); and Gambel sparrow, 2 sheathed forms (117 $\mu \times 4 \mu$) in one bird, 22 unsheathed (88 $\mu \times 4 \mu$) in a second bird, and 27 unsheathed forms (87 $\mu \times 3 \mu$) in a third bird.

Spirogregarina fusiformis n. g., n. sp.
(Figs. 1–3)

Morphology: Elongate, spindle-shaped organism (Fig. 1), greatest width in center of cell where nucleus located. Body form in most specimens cylindrical; some individuals show flattening of extremities manifested by a twisting across itself of that portion of body. Most parasites straight in thick or thin parts of smear. On one slide an organism was found bent into half-moon shape. Ends of parasite flare to produce bulbous, knob-like terminus at each end (Figs. 1, 3). Outer surface of these expanded ends sometimes seems indented forming half-moon shaped flattened area (Fig. 3). Thus, parasite tapers gradually from middle nuclear zone of uniform width to neck-like constriction short distance from bulbous end, then flares out slightly towards rounded ends of cell.

Outer surface of parasite appears stiff but elastic suggesting presence of pellicle. Surface lined (grooved?) with light appearing, criss-cross bands of varying width running length of parasite in spiral arrangement. At least one spirally arranged band in clockwise direction and one in counter-clockwise direction.

Nucleus rounded and very compact in some individuals (Fig. 1); in others appears flattened. Frequently irregular in shape. Chromatin in dense globular masses which merge into each

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Fig. 6. Intra-leucocytic parasite in a monocyte from a common house finch.
Fig. 7. Free intra-leucocytic parasite from a common house finch.
Fig. 8. Intra-leucocytic parasite in a small lymphocyte from a common house finch.
Fig. 9. Intra-leucocytic parasite in a medium-sized lymphocyte from a western mockingbird.
Fig. 10. Intra-leucocytic parasite in a large lymphocyte from a western mockingbird.
Figs. 11 and 12. *Hepatozoon* in thrombocytes from an Audubon warbler.
Fig. 13. *Hepatozoon* in a small lymphocyte from a San Diego titmouse.
Fig. 14. *Hepatozoon* in a small lymphocyte from another San Diego titmouse.
Fig. 15. *Hepatozoon* in a small lymphocyte from a black-headed grosbeak.
Fig. 16. *Hepatozoon* in a medium-sized lymphocyte from a California thrasher.
Fig. 17. *Hepatozoon* in a small lymphocyte from a common house finch.
Fig. 18. *Hepatozoon* in a small lymphocyte from a pallid wren-tit.

As seen on blood smears stained with Jenner-Giemsa, the nuclei of Figs. 1 and 2 appear purplish-red like the finer cytoplasmic granules while the coarser cytoplasmic granules around the nucleus (Fig. 2) and near the cell extremities are dark purplish-red. The striations vary from colorless to pink while the inclusion bodies have a reddish purple or reddish blue cast. The finely granular cytoplasm is reddish pink.

The black nuclei of the parasites in Figs. 4 through 10 are pale pink. The cytoplasmic granules vary from pink to purplish red and in some cells are almost black. The cytoplasm varies from pale blue to dark blue. The mottled appearance of the cytoplasm of the parasites, Figs. 4 through 18, is illustrated here with a fine stippling which does not represent the very fine cytoplasmic granulation.

The middle, band-like nuclear areas of the parasites in Figs. 11 through 18 vary from pale pink to purplish-red. The cytoplasm appears pale blue or gray-blue. The granules outside the nucleus are pale pink in some parasites or reddish purple to black in others. The vacuoles are usually colorless but appear pale blue in dark preparations.

other giving nucleus closely beaded appearance. In at least two specimens no distinct purplish-red staining nucleus found. In a few parasites, nucleus showed distinct bluish-staining nucleolus. Nucleus of number of specimens embedded in granular mass of material of similar structure to that of nucleus (Fig. 2). This granular mass tended to surround nucleus. Frequently, nucleus partly hidden by large, deep bluish-staining, oval bodies in cytoplasm.

Cytoplasm of parasite finely granular giving distinctly homogeneous appearance to lightly-stained individuals. Sometimes small unequal-sized, clear vacuoles in cytoplasm with especially thick membranes. In some cells, a number of coarse, deep purple-red staining granules distributed toward either extremity of cell, around nucleus, or along lighter staining spiral bands. In a few parasites these larger granules found throughout cytoplasm. In most parasites cytoplasm contained deep bluish-staining, oval masses (Fig. 1), so numerous as to completely fill central cytoplasm of cell. Oval masses sometimes showed two distinct parts, outer lighter homogeneous zone and central darker, less homogeneous area. Such structures similar in size and shape to red blood cells of host. Suggests source of food of parasite although structure of all parasites does not clearly indicate nature, or possibility, of ingestion apparatus.

Size: Average length of ten parasites chosen at random: 106.6 μ (96.3 μ–113.7 μ). Diameter at widest central point averaged 4.7 μ. Narrowest point of constriction before the end averaged 1.2 μ and diameter of the expanded end averaged 1.8 μ.

Host: The parasite was found in one adult rock wren, *Salpinctes obsoletus*, collected July 11, 1938, on San Nicolas Island off Southern California by Professor Loye H. Miller of the University of California at Los Angeles. There were 56 parasites seen on one slide and 22 specimens on a second slide from the same bird. Type material is in the private slide collection of the authors.

This parasite is similar to *Sergentella hominis* Brumpt (Brumpt, 1927) in its spindle-like form and spiral lines but differs from it in its smaller, oval nucleus, knobbed extremities and much larger size. From the visible structure of *Spirogregarina fusiformis* in stained smears, it seems most closely related to certain developmental stages of haemogregarines. Because of its distinctive form, it is impossible to identify it with any known genus of the Sporozoa.

MULTIPLE INFECTIONS

As is evident from a glance at Table 1 comparing number infected and total types of parasites indicated, many birds harbored more than one parasite. For those cases in which only one infected bird was found, the table indicates the types of parasites. The organisms occurring in mixed infections were *Trypanosoma* (T), *Haemoproteus* (H), *Plasmodium* (P), *Leucocytozoon* (L), intra-leucocytic parasite (I), and microfilarial worms (M). By using the letters indicated above, the complete figures for the birds with two or more genera of parasites is indicated as follows: THLM in 1 California spotted owl and 1 russet backed thrush; THL in 1 Bullock oriole, 5 black-headed grosbeaks, and 1 Gambel sparrow; TPL in 1 Anthony green heron; HLM in 1 common mallard and 1 Gambel sparrow; THP in 1 western white-winged dove; TLM in 1 evening grosbeak; TH in 2 hooded orioles, 2 Bullock orioles, 1 western tanager and 1 Gambel sparrow; TL in 2 American pintail, 1 Brewer blackbird and 1 evening grosbeak; HP in 1 hooded oriole and 2 common house finches; HL in 4 western mourning doves, 1 western tanager, 5 black-headed grosbeaks and 4 Gambel sparrows; HM in 1 hooded oriole and 2 Gambel sparrows; PL in 2 California jays, 1 western mockingbird and 2 common house finches; PI in 1 dwarf cowbird; and LM in 1 red-breasted merganser and 1 western robin.

It is interesting to note that the most common double infection was with *Haemoproteus* and *Leucocytozoon* (14 cases), whereas the commonest triple infection involved *Trypanosoma, Haemoproteus* and *Leucocytozoon* (7 cases).

Of 26 infected western mourning doves, 15 were infected with *Haemoproteus columbae,* 1 harbored *Haemoproteus sacharovi,* and 10 were infected with both species. Of 4 infected western white-winged doves, 3 harbored *Haemoproteus columbae,* and 1 was infected with *H. columbae* and *H. sacharovi.*

SUMMARY

In the present survey, 1,525 birds of 112 species and subspecies were examined. Three hundred fifty-seven individuals or 23.4 per cent, representing 54 species or 48.2 per cent, were infected. Trypanosomes were found in 42 birds of 22 species including the following new host records: Anthony green heron, American pintail, western white-winged dove, California spotted owl, eastern flicker, western flycatcher, russet-backed thrush, yellow warbler, Macgillivray warbler, pileolated warbler, hooded oriole, Bullock oriole, Brewer blackbird, dwarf cowbird, western tanager, black-headed grosbeak, evening grosbeak, California purple finch, willow goldfinch, and Gambel sparrow.

Haemoproteus was observed in 159 birds of 21 species including the following new host records: American pintail, San Quintín quail, Gambel quail, mountain quail, band-tailed pigeon, western white-winged dove, spotted screech owl, California spotted owl, russet-backed thrush, Audubon warbler, black-throated gray warbler, hooded oriole, Bullock oriole, western tanager, black-headed grosbeak, and San Clemente house finch.

Plasmodium was found in 71 birds of 11 species including the following new host records: Anthony green heron, western white-winged dove, hooded oriole, dwarf cowbird, California towhee and San Diego song sparrow.

Leucocytozoon was found in 123 birds of 33 species including the following new host records: Anthony green heron, cackling goose, white-fronted goose, redhead, sharp-shinned hawk, band-tailed pigeon, western mourning dove, Chinese spotted dove, California spotted owl, western mockingbird, California thrasher, western robin, hermit thrush, russet-backed thrush, orange-crowned warbler, Calaveras warbler, olive warbler, yellow warbler, Audubon warbler, Bullock oriole, Brewer blackbird, dwarf cowbird, western tanager, black-headed grosbeak, lazuli bunting, evening grosbeak, willow goldfinch and Gambel sparrow.

Hepatozoon was found in the following new hosts: 2 San Diego titmice, 1 pallid wren-tit, 1 California thrasher, 1 Audubon warbler, 1 black-headed grosbeak and 2 common house finches.

Intra-leucocytic parasites were found in 3 western mockingbirds, 1 English sparrow, 1 dwarf cowbird and 2 common house finches. The first three birds represent new host records.

Microfilarial worms were found in 13 birds of 10 species including the following new hosts: red-breasted merganser, California spotted owl, western robin, russet-backed thrush, pileolated warbler, hooded oriole, black-headed grosbeak, evening grosbeak and Gambel sparrow.

A new genus and species of parasite, *Spirogregarina fusiformis,* was reported from the rock wren.

No parasites were observed in 57 species represented by 98 birds. Mixed infections were found in 52 birds of 20 species, 38 individuals were infected with two genera of parasites, 14 with three. Two common house finches were infected with both *Plasmodium cathemerium* and *P. relictum.*

REFERENCES

BRUMPT, E. 1927 Précis de Parasitologie. 4th Ed., Masson & Cie, Paris.
Check-list of North American Birds. 1931 4th Ed. American Ornith. Union.
HEWITT, R. 1940 Studies on blood protozoa obtained from Mexican wild birds. J. Parasitol. **26**: 287–295.
WENYON, C. M. 1926 Protozoology. Wm. Wood & Co., New York.
WOOD, F. D. AND WOOD, S. F. 1937 Occurrence of haematozoa in some California birds and mammals. J. Parasitol. **23**: 197–202.

FLAGELLATE PARASITES OF MOSQUITOES WITH SPECIAL REFERENCE TO *CRITHIDIA FASCICULATA* LÉGER, 1902*

F. G. WALLACE
Department of Zoology, University of Minnesota

Since Ross' original discovery of flagellate parasites in the intestines of mosquitoes in 1898 numerous investigators have found parasites of this type in many parts of the world. As the earlier records are summarized by Novy, MacNeal, and Torrey (1907), Wenyon (1926), and Speer (1927) they will not be listed here in full but will be referred to only as they bear on questions of nomenclature and biology. The records made since Wenyon's and Speer's compilations are listed in Table 1.

TABLE 1.—*Recent records of flagellate parasites found in mosquitoes*

Date	Author	Locality	Host species	Per cent infected	Name of parasite
1926	Hindle & Patton	China	*Culex fatigans*		Undetermined
1926	Noguchi & Tilden	Georgia	*A. quadrimaculatus*		*H. culicidarum*
"	"	New Jersey	*Culex pipiens*		"
1928	Shakhov	Russia	*A. maculipennis*	5 to 7	*C. fasciculata*
1930	Missiroli	Italy	*A. maculipennis*	2.3	*H. culicis* (= *C. fasciculata*)
1930	de Boissezon	France	*Culex pipiens*	(1 specimen)	*C. fasciculata*
1930	Taylor	Nigeria	*Lutzia tigripes*	(1 specimen)	Undetermined
1933	Feng	China	*A. jeyporensis*	3.3	*H. culicis* (= *C. fasciculata*)
			A. hyrcanus sinensis	12.5	*H. culicis* (= *C. fasciculata*)
1935	Barber & Rice	Macedonia	*A. maculipennis*		"Flagellates including *C. fasciculata*"
1941	Ayroza & Coutinho	Brazil	*A. oswaldoi*	7.35	
			A. triannulatus davisi	3.44	*H. pessoai*

(*A.* = *Anopheles* throughout)

NOMENCLATURE

The first technical name given to a mosquito flagellate was *Crithidia fasciculata* Léger, 1902. Léger described the species as small flagellates, 4 μ to 10 μ long, shaped like a barley corn, truncate at the anterior end and, according to one figure, with a funnel-like depression at the anterior end. In addition to these forms and connected to them by a series of intermediate stages he found more elongate forms (8 μ to 14 μ) which might have had an undulating membrane; "– sur l'un des côtés, le corps, plus aminci et à contour ondulé montre comme un rudiment de membrane ondulante." His casual sketches leave doubt as to whether an undulating membrane was actually seen or not.

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Novy, MacNeal, and Torrey (1907) made an extensive study of mosquito flagellates redescribing *C. fasciculata* and isolating it in pure culture. They discovered and isolated a second species, *Trypanosoma (Herpetomonas) culicis,* which was longer and provided with a short undulating membrane, and suggested that Léger may have been dealing with a mixed infection.

Patton (1908), on the basis of his life history studies of insect flagellates, thoroughly confused the nomenclature of these forms. Assuming that all short truncate flagellates are "young" forms while long slender ones are "adults," a thesis which he fails to support by experimental evidence, he says that, even though Léger may have seen two species, his *Crithidia fasciculata* can not be based on the short or young form but that the longer forms, presumably with the undulating membrane, must be accepted as typical of the species. He thought that Novy, MacNeal, and Torrey's *C. fasciculata,* lacking a membrane, should be called *Herpetomonas culicis,* while their *H. culicis,* having an undulating membrane, should be called a *Crithidia.* Patton (1909) says: "A reference to a recent paper by Novy, MacNeal, and Torrey will shew that by following Léger's description of the genus they have named a true Crithidia a Herpetomonas." This is a curious interpretation of taxonomic rules as a "true Crithidia" should, presumably, be based on the original description.

Woodcock (1914), believing that Léger had seen only one species, thought that *C. fasciculata* really had an undulating membrane. The organisms found by Novy, MacNeal and Torrey and by himself, and lacking a membrane should, he thought, receive a different name, *Leptomonas fasciculata.* Speer (1926) pointed out that the use of the same specific name in two closely related genera is contrary to the international code and renamed Novy's form *L. michiganensis.* Subsequent authors have all followed Patton's and Woodcock's lead in assuming that the type of the genus *Crithidia* had an undulating membrane.

Actually Léger, in his original designation of *Crithidia fasciculata,* gave a distinctive and recognizable description of a short truncate membraneless flagellate which attaches in masses to the walls of the hind gut. The existence of such flagellates in mosquitoes has been confirmed, in the study of parasites from insects and from cultures, by many authors; most critically by Novy et al (1907), Fraenkel (1909, working with Novy's strain), Woodcock (1913), Nöller (1917), Schulz (1924), Shakhov (1928), Thomson (1930, Noguchi's strain), Lwoff and Lwoff (1931), and the present author. None of these workers found an undulating membrane in any stage. The present author has studied these parasites in naturally infected mosquitoes, in cultures, and in mosquitoes artificially infected with pure cultures. Léger's reference to longer forms which may have had an undulating membrane appears, in his paper, after the description of the membraneless "barleycorn" type which was clearly the form meant to be called *Crithidia fasciculata.* If he actually saw an undulating membrane, a point that is not indisputably made, it is much more probable that he had a second species than that he had a species identical with the forms found and studied many times since, except for the possession of a membrane. His reference to intergrading forms can not be accepted as indicating the unity of these organisms as, while there may be an overlapping in size ranges, the presence or absence of a membrane is a sharp and definite character.

Novy, MacNeal and Torrey's description of *Herpetomonas culicis* shows that there is a flagellate in mosquitoes with a membrane and distinct from *Crithidia*

fasciculata in the host and in culture. Mezincesco (1908), Missiroli (1930) and the present author have confirmed this to the extent of finding two distinct types in mosquitoes; one short, truncate, membraneless, and attached to the walls of the hind gut and the other longer, provided with a membrane, and free in the mid gut. Ayroza and Coutinho (1941) made a careful study of *Herpetomonas pessoai* from the mid gut of anophelines which has not only stages with a short undulating membrane but trypanosome-like stages with a post-nuclear kinetoplast and a long undulating membrane.

The rediscovery of flagellates identical with Léger's *C. fasciculata* and lacking the membrane in all stages, the known existence of other species with the membrane in mosquitoes, and the discrediting of the life cycle of *Herpetomonas culicis* (= *C. fasciculata*) as described by Patton (which will be discussed below), remove the basis for stating that *Crithidia fasciculata* has an undulating membrane.

Thomson (1930) in describing Noguchi's strain of *H. culicidarum*, which, as pointed out by Nöller (1928) is identical with *C. fasciculata*, noted the funnel-like depression in the anterior end, which was suggested in one of Léger's sketches, and the whirling motion of the flagellum. Lwoff and Lwoff (1931), using Nöller's strain of *C. fasciculata*, pointed out these features and discovered 12 spiral lines on the surface which were stained by mitochondrial techniques. They erected the new subgenus *Strigomonas* in the genus *Leptomonas*, for this form but in view of the above arguments for stating that *Crithidia fasciculata* has no undulating membrane *Strigomonas* becomes a synonym of *Crithidia*.

We may, then, characterize *Crithidia fasciculata* as short flagellates rounded or pointed posteriorly, truncate and with a funnel-shaped depression anteriorly. Nucleus at or behind middle of cell. Kinetoplast slightly anterolateral from nucleus. Axoneme extending just under cell membrane to anterior end where it emerges excentrically. Cell membrane with about 12 spiral striae shown by mitochondrial technique. Organisms tend to attach themselves in clusters or to walls of host intestine. Parasites of hind gut of mosquitoes.

Members of the genus *Crithidia* differ from *Leptomonas* in the short anteriorly truncate shape, the funnel-like depression at the anterior end, the median or posteriorly situated kinetoplast, and the long axoneme emerging as the flagellum excentrically from the anterior end. They differ from members of the genus *Herpetomonas* in the same respects and in the complete absence of the undulating membrane.

It is with reluctance that the author proposes to discontinue the long established use of the name *Crithidia* as a generic name for trypanosomids with the short undulating membrane or as a term descriptive of such stages of members of the genera *Herpetomonas* and *Trypanosoma*, but as this use of the term appears to be based on a misconception of the type species and its description we have no choice.

LIST OF NAMES USED FOR MOSQUITO FLAGELLATES

Crithidia fasciculata Léger 1902

Synonyms: *Herpetomonas culicis* Novy, MacNeal and Torrey, 1907, as used by Patton and others

Leptomonas fasciculata Woodcock, 1914

Leptomonas michiganensis Speer, 1926

Herpetomonas culicidarum Noguchi and Tilden, 1926

Crithidia anophelis Missiroli, 1930
Leptomonas (*Strigomonas*) *fasciculata* Lwoff and Lwoff, 1931
Herpetomonas algeriense Sergent and Sergent, 1906
Herpetomonas culicis Novy, MacNeal and Torrey, 1907
Herpetomonas myzomyiae Drbholav, 1925 (insufficiently described)
Herpetomonas pessoai Ayroza and Coutinho, 1941

MATERIALS AND METHODS

Rearing mosquitoes: A colony of *Culex pipiens,* established from hibernating females among which the flagellate infections studied were found, has been kept through six generations. The species was determined by study of male genitalia and larval structure. The wild-caught females were fed raisins and oviposited without a blood meal. Neither these nor subsequent generations would bite human beings though offered many opportunities. The cage-reared females were fed on day-old mice and canaries as well as on soaked raisins. Larvae were reared in finger bowls and fed a variety of food including bread crumbs, dog biscuit, powdered milk, and yeast. Eighty-five adults and many larvae from the colony were examined as controls but none was found infected with protozoa.

A colony of *Aëdes aegypti,* originally obtained from the Army Medical School, Washington, and reared according to the usual methods, was also used for experimental infections.

Dissection of mosquitoes: The entire intestine of the mosquito was drawn out as in examination of *Plasmodium* oöcysts and mounted with care to avoid cover-glass pressure. Infected intestines were studied in toto and then smeared for staining with Giemsa's stain.

Isolation of pure cultures: When culturing was to be attempted each mosquito was anesthetized, the legs and wings were cut off, and the body was dropped for two minutes in a dish of 0.6 per cent $HgCl_2$ to which a drop of tergitol had been added. After this bath it was washed successively in six tubes of sterile saline, the transfers being made with a flamed wire loop. The dissection and examination were done with sterile precautions, the needles being washed in alcohol and then sterile saline, and the slides and covers flamed. Infected intestines were teased in sterile broth and the suspension streaked on plates and planted in tubes.

Plates were made by adding 2 cc of human blood cells, left after plasma is drawn off, or 3 cc of rabbit blood to 10 cc of Difco nutrient agar adjusted to litmus neutrality. In tubes, N.N.N., Difco nutrient agar with or without blood, or neutral peptone broth with or without blood were used. The best growth was obtained on N.N.N. tubes to which 1 cc of peptone broth each was added but abundant growth occurred on all media used. All cultures were kept at room temperature.

OCCURRENCE AND ISOLATION

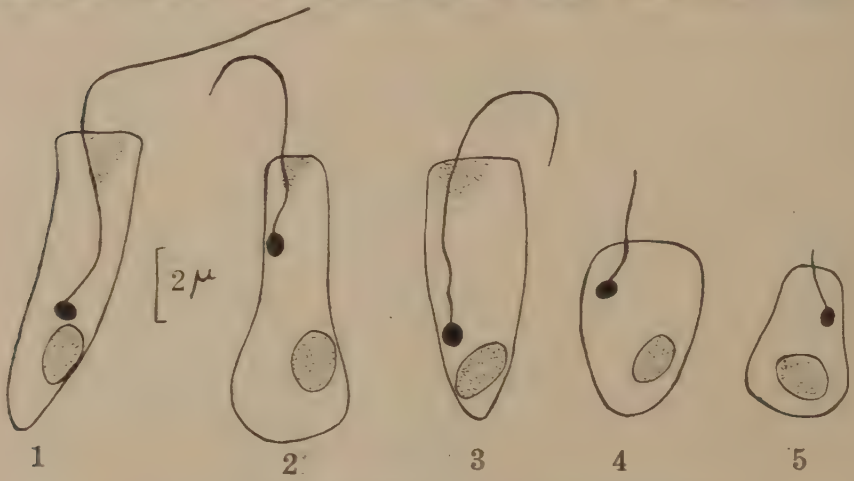
Among 18 *Culex pipiens* collected from a cave on the bank of the Mississippi in Saint Paul, Minn., in January 1942, 8 were found infected with flagellates. Two were infected with long slender forms which were free in the hind and mid guts. The difference between these and *Crithidia fasciculata* as seen in fresh mounts of the entire intestine, is striking. The organisms were studied in fresh and Giemsa-stained smears and identified as *Herpetomonas culicis* Novy, MacNeal and Torrey.

Six of the mosquitoes were infected with short flagellates attached in enormous numbers to the walls of the hind gut which were identified as *Crithidia fasciculata* Léger. Cultures were made and a number of strains persisted through several transplants, though heavily contaminated with bacteria. Attempts to isolate these strains failed and they died out.

On March 19, one specimen among 30 *Culex pipiens* from the same cave was found infected with *Crithidia*. The organisms were plated and planted in a tube of N.N.N. medium. The plates failed to show growth but a rich culture of flagellates mixed with yeast appeared in the tube. This mixed culture grew abundantly through five transplants. After a number of attempts pure colonies were obtained on blood agar plates after 6 days' incubation and the strain thus isolated has been maintained since.

MORPHOLOGY

Crithidia fasciculata in the active or nectomonad form is from 6 μ to 8 μ long and 2 μ to 3 μ wide. The shape, while variable, exhibits certain features which



FIGS. 1 to 5. *Crithidia fasciculata* as seen in Giemsa stained smears.

distinguish it from *Herpetomonas culicis* and other flagellates. The anterior end is truncate, sometimes with a very slight narrowing behind it so that the anterior tip is vase-shaped. There is a funnel-shaped depression in the anterior end. Usually the widest point is at about the middle of the length, and the posterior end tapers to a point that is slightly rounded off. Sometimes the posterior part is inflated and the anterior half cylindrical (Figs. 1–5).

In Giemsa-stained smears the nucleus is in the posterior half of the body. The kinetoplast is close in front of it, at or posterior to the middle of the cell and close to one side. From it the axoneme extends forward, near the surface to the anterior end where the flagellum emerges near one side (Fig. 6). There is no trace of an undulating membrane. This has been verified by dark-field study of living forms and careful study of living and stained slides. The author has confirmed the presence of about 12 spiral striae on the surface of the cell, first observed by Lwoff and Lwoff and shown by Altman's fuchsin technique.

The small rounded forms or haptomonads, which are attached to surfaces are 3 μ to 4 μ long and 2 μ to 4 μ wide. The nucleus, kinetoplast, and axoneme are seen in varying positions and the free flagellum is absent or very short. These forms are seen both in culture and in the mosquito. In culture they attach themselves to any surface; to the surface film of an uncovered drop, to the glass, or to an air bubble. Novy, MacNeal and Torrey called attention to their tendency to cluster around bubbles.

In the host all the flagellates are normally in the motionless haptomonad form and attached to the gut wall. When the intestine is very carefully drawn out and not pressed by the cover glass, that is seen to be the case. As the preparation is squeezed, more and more become free and swim about. If the intestine is broken so that the organisms escape into the saline, they all swim actively. This was noted by Ross in his pioneer work.

Patton (1907 and 1912) describes a life history for *Crithidia fasciculata* with rounded attached preflagellate stages in larvae, flagellate or adult stages in pupae and adults, and postflagellate stages in the rectum of the adult. The cycle was not worked out experimentally but by arranging the stages found in natural infections in an arbitrary sequence. There is no morphological difference between his preflagellate and postflagellate stages. His observations can be explained equally well on the basis of fortuitous variations in behavior as a result of differences in treatment during observation. In the experimental infections described below, no such sequence of stages could be found in larval and adult infections or in infections of varying duration. The majority of the organisms were always in the haptomonad or attached form until disturbed when they would become active. The only difference noted between infections of 50 minutes and 22 days' duration was in the number present.

BIOLOGY

The basic question regarding any haemoflagellate of blood sucking insects is whether it is a stage in the development of a parasite of vertebrates or is a parasite of the insect alone. While many workers have suggested, and a few have claimed experimental evidence, that flagellates of mosquitoes were stages in the development of parasites of birds or mammals, we have no acceptable proof that such is the case. The claims of Schaudinn (1904), the Sergents (1905), Laveran and Franchini (1914), and Fantham and Porter (1915) are discussed in extenso by Novy, MacNeal and Torrey and by Wenyon and need not be reconsidered here.

On the other hand observations by the Sergents (1906) and Patton (1912) provide circumstantial evidence that infection with certain species is acquired in the larval stage. The Sergents found natural infections of *Herpetomonas algeriensis* in *Culex* and *Aëdes aegypti* which were reared from larvae in the laboratory. Patton found approximately 95 per cent of the larvae and pupae of *Culex fatigans* in a well at Madras infected with *H. culicis* (= *Crithidia fasciculata*) and concluded that the source of infection must have been the water. Hitherto no one has infected mosquitoes experimentally.

ATTEMPTS TO INFECT LARVAE

On April 16 a number of second and third stage *Aëdes* and *Culex* larvae were dropped into a rich broth culture of *Crithidia* and after two minutes removed to

clean water. Organisms were seen in the gut of dissected larvae immediately afterward. Eighteen adults which emerged eight to twelve days later were uninfected.

On May 1 a number of fourth stage larvae of *Culex* were immersed in a broth culture for two minutes and then transferred to a bowl of clean water to which the culture was added. A larva dissected immediately after the immersion had numerous flagellates in the fore gut. One dissected after 50 minutes showed numerous flagellates in the mid gut, many being attached side by side to the gut wall as in the natural infections of adults. The next day a pupa which had formed in the meantime was found negative but two out of three larvae examined showed many organisms lined up along the walls of the mid and hind guts. A few living *Crithidia* were still in the water. Twelve adults which emerged in from four to six days were negative.

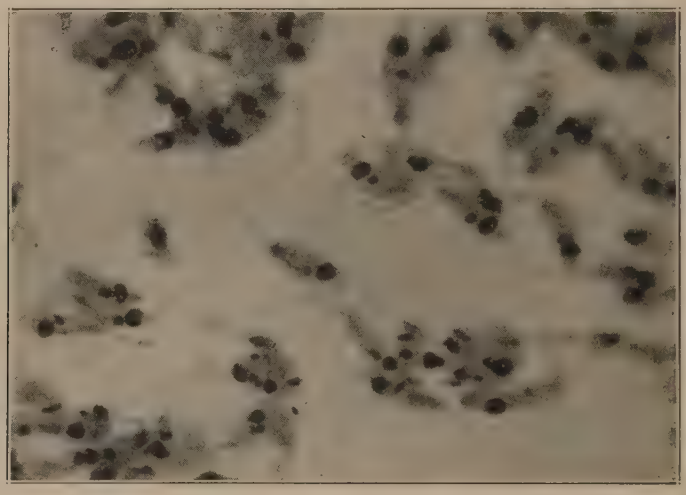


FIG. 6. Photomicrograph of *Crithidia fasciculata* stained with Giemsa's stain.

On May 11 and May 17 rich broth cultures of *Crithidia* were poured into a bowl of second stage *Culex* larvae. Fourteen adults which emerged between June 2 and 9 were negative.

The temporary infections of larvae indicate that the method of administering the protozoa was effective. The failure of the flagellates to remain into the adult stage can not be explained, though an abundant bacterial flora in the larvae may be detrimental to the protozoa. It is possible that with larvae reared under different conditions the parasites acquired in that stage will persist into the adult stage.

INFECTION OF ADULT MOSQUITOES

Adult *Culex pipiens* and *Aëdes aegypti* can be readily infected with *Crithidia fasciculata* in the laboratory. Mosquitoes are put in lantern globes with netting tops and kept in a moist cabinet without food or water for 24 hours. Then a sterile cotton swab is dipped in a culture of *Crithidia* and is either offered to the mosquitoes one by one, if patience permits, or is simply left in the lantern globe until dry. A

drop of sugar solution on the swab appears to make the culture more acceptable. All of 15 mosquitoes which were known to have fed on the culture became infected. Of 38 mosquitoes exposed to the infection by having opportunity to feed on the culture (though no observations were made as to whether they actually drank or not), 20 became infected. Mosquitoes were examined as early as three days and as late as 22 days after infection. In the three-day infection parasites were abundant and in thick clusters in the hind gut but they did not cover the entire surface. In all infections examined after six days or more the flagellates were as abundant as in the natural infections. They cover the entire lining of the hind gut, rectal pouch, and rectal papillae like a carpet and the lumen is packed with clusters (Fig. 7). It is suggested that in laboratories where mosquito colonies are kept such demonstra-

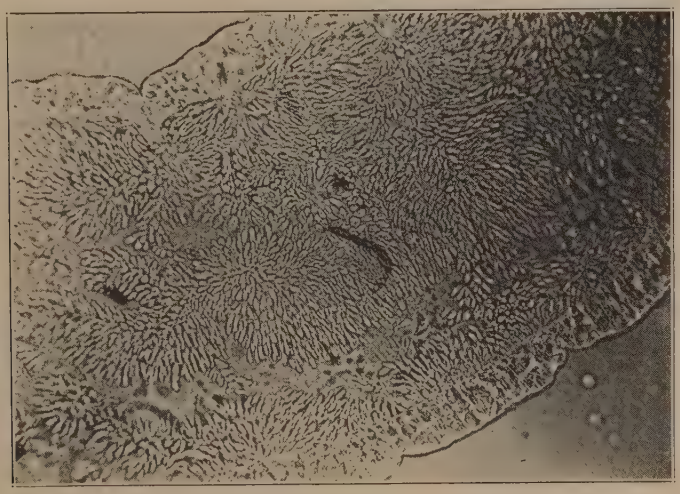


FIG. 7. Photomicrograph of fresh preparation of hind gut of mosquito infected with *Crithidia fasciculata*.

tions of infected mosquitoes are very easily prepared and are a striking supplement to laboratory work on insect born flagellates.

TRANSMISSION

A number of *Culex pipiens* which had had opportunity to feed on a *Crithidia* culture were caged in clean lantern globes with uninfected mosquitoes, which were marked by cutting off the end of the wing. This mark was easy to recognize at the end of the experiment.

In one globe three infected females were placed with six males and six females, all uninfected. Twenty days later the one of the originally exposed specimens still alive was found infected. Five males and five females of the test group were infected, while one of each sex was negative.

In another globe nine exposed males were caged with six uninfected males and six uninfected females. Twenty days later the insects remaining were examined. Five of the originally exposed males were infected and three were negative. Of the

test males two were negative and two positive and of the females four were positive and two negative.

This experiment shows that in the limited confines of a lantern globe the infection is readily passed from one mosquito to another. This presumably occurs through the ingestion of feces as numerous droplets are found on the glass surface but the exact mechanism of transfer was not determined. There is no evidence and little probability that fecal contamination in nature, unless mosquitoes are breeding in a small space, is a factor in the natural dissemination of the parasite.

SUMMARY

1. *Crithidia fasciculata* Léger, 1902, type of the genus *Crithidia,* has no undulating membrane.

2. Patton's life cycle of *Herpetomonas culicis* (= *C. fasciculata*) involving preflagellate, flagellate, and postflagellate stages is erroneous.

3. The parasites are normally attached in the small rounded form to the gut lining but become free when disturbed.

4. Transitory infections were produced in larval mosquitoes by feeding cultures of *Crithidia* but the organisms did not persist into the adult stage.

5. Adult mosquitoes are easily infected by feeding them cultures.

6. *Crithidia fasciculata* is transferred from one mosquito to another when the insects are confined in lantern globes.

REFERENCES

AYROZA GALVÃO, A. L. E J. O. COUTINHO 1941 Nota sobre um flagelado parasita de anofelinos do sub-genero "Nyssorhynchus," "Herpetomonas Pessoai" n. sp. Rev. Brasil. Biol. **1**: 311–319.

BARBER, M. A. AND J. B. RICE 1935 Malaria studies in Greece. The malaria infection rate in nature and in the laboratory of certain species of Anopheles of East Macedonia. Ann. Trop. Med. and Parasitol. **29**: 329–348.

BOISSEZON, P. DE 1930 La biologie et de l'histophysiologie de Culex pipiens. Arch. Zool. Expér. et Gén. **70**: 281–431.

DRBOHLAV, J. J. 1925 Studies on the relation of insect herpetomonad and crithidial flagellates to leishmaniasis. 1. Inoculation of mammals with insect flagellates. Am. J. Hyg. **5**: 580; 599–611.

FANTHAM, H. B. AND A. PORTER 1915 Some experimental researches on induced herpetomoniasis in birds. Ann. Trop. Med. and Parasitol. **9**: 543–558.

FENG, LAN CHOU 1933 Some parasites of mosquitoes and flies found in China. Lingnan Sc. J. **12** Suppl: 23–31.

FRAENKEL, C. 1909 Beobachtungen an Crithidia fasciculata. Hyg. Rundschau **19**: 57–58.

HINDLE, E. AND W. S. PATTON 1926 Reports from the Royal Society's kala azar commission in China, No. 4. Proc. Roy. Soc. B **100**: 385.

LAVERAN, A. AND G. FRANCHINI 1914 Infections de mammifères par des flagellés d'invertebrés. Bull. Soc. Path. Exot. **7**: 605–612.

LÉGER, L. 1902 Sur un flagelle parasite de l'Anopheles maculipennis. Compt. Rend. Soc. Biol., Paris **54**: 354–356.

LWOFF, MARGUERITE AND ANDRE 1931 Protistologica 26. Recherches sur la morphologie de Leptomonas oncopelti, Noguchi et Tilden, et Leptomonas fasciculata Novy, MacNeal et Torrey. Arch. Zool. Exper. et Gen. Notes et Rev. **71**: 21–37.

MEZINCESCO, D. 1908 Les trypanosomes des moustiques et leurs relations avec les haemoproteus des oiseaux. Compt. Rend. Soc. Biol., Paris **64**: 975–976.

MISSIROLI, A. 1930 Richerchi sui flagellati che si riscontrano nell' "Anopheles maculipennis." Riv. Malariol. **9**: 111–119.

NOGUCHI, HIDEYO AND E. B. TILDEN 1926 Comparative studies of herpetomonads and leishmanias. 1. Cultivation of herpetomonads from insects and plants. J. Exper. Med. **44**: 307–325.

NÖLLER, W. 1917 Blut und Insecten Flagellaten Züchtung auf' Platten. Arch. Schiffs- u. Tropen-Hyg. **21**: 53–94.

————1928 Die Zuchtung der parasitischen Protozoen. Handbuch der Pathogenen Protozoen. J. A. Barth, Leipzig.

NOVY, F. G., W. J. MACNEAL AND H. N. TORREY 1907 The trypanosomes of mosquitoes and other insects. J. Infec. Dis. **4**: 223–276.

PATTON, W. S. 1907 Preliminary note on the life cycle of a species of Herpetomonas found in Culex pipiens. Brit. Med. J. (July 13): 78–80.

————1908 The life cycle of a species of Crithidia parasitic in the intestinal tract of Gerris fossarum. Fabr. Arch. Protistenk. **12**: 131–146.

————1909 The life cycle of a species of Crithidia parasitic in the intestinal tracts of Tabanus hilarius and Tabanus sp. Arch. Protistenk. **15**: 333–362.

————1912 Studies on the flagellates of the genera Herpetomonas, Crithidia, and Rhynchoidomonas. I. The Morphology and life history of Herpetomonas culicis Novy, MacNeal, and Torrey. Sci. Mem. Med. and San. Dept. Gov. India, New Series No. 57: 1–21.

SCHAUDINN, F. 1904 Generations- und Wirtswechsel bei Trypanosoma und Spirochaeta (Vorlaufige Mitteilung). Arb. K. Gesundhtsamte, Berlin **20**: 387–439.

SCHULZ, M. 1924 Die Kernteilung von Leptomonas fasciculata nebst einen vergleich mit der Kernteilung bei Leishmania donovani. Arch. Protistenk. **49**: 216–236.

SERGENT, EDM. ET ET. 1905 Hemamibes des oiseaux et moustiques: "Générations alternantes" de Schaudinn. Compt. Rend. Soc. Biol., Paris **58**: 57–59.

———— ET ————1906 Sur un flagelle nouveau de l'intestin des Culex et des Stegomyia, Herpetomonas algeriense: Sur un autre flagelle et sur des spirochaetes de l'intestine des larves de moustiques. Compt. Rend. Soc. Biol., Paris **60**: 291–293.

SHAKHOV, S. D. 1928 On the flagellates from the intestines of mosquitoes (in Russian). Vestnik Microbiol., Epidemiol. i. Parazitol. (Rev. Microbiol., Epidemiol. et Parasitol.) **7**: 421–422.

SPEER, A. J. 1926 *Leptomonas michiganensis*, a new name for the American *Crithidia fasciculata*. J. Parasitol. **13**: 88.

————1927 Compendium of the parasites of mosquitoes (Culicidae). Bull. Hyg. Lab., U. S. Pub. Health Serv. 146.

TAYLOR, A. W. 1930 The domestic mosquitoes of Gadau, Northern Nigeria, and their relation to malaria and filariasis. Ann. Trop. Med. and Parasitol. **24**: 425–435.

THOMSON, J. G. 1930 Cultivation of Herpetomonas culicidarium on ordinary broth and nutrient agar: morphology of the flagellate. Proc. Roy. Soc. Trop. Med. and Hyg. **24**: 5.

WENYON, C. M. 1926 Protozoology. 2 vol. London.

WOODCOCK, H. M. 1914 Further remarks on the flagellate parasites of Culex. Is there a generic type, Crithidia? Zool. Anz. **44**: 26–33.

FOOD HABITS AND INTENSITY OF COCCIDIAN INFECTION IN NATIVE VALLEY QUAIL IN CALIFORNIA[1]

CARLTON M. HERMAN[2] AND JOHN E. CHATTIN
California Division of Fish and Game
AND
ROY W. SAARNI
U. S. Forest Service

Examination of fecal samples from large series of California quail (*Lophortyx californica*), taken in various areas in California during 1941, showed a high incidence of coccidian infection. Herman and Jankiewicz (1942) have demonstrated that the infection reduces to a minimum in the absence of material contaminated with ripe coccidian oöcysts. Herman, Jankiewicz and Saarni (1942) showed further that, under natural conditions, the intensity of infection in individual quail tends to fluctuate, probably decreasing as the parasites are eliminated and increasing with the repeated ingestion of oöcysts.

A possible relationship between the intensity of coccidian infection and food habits was observed from data on fecal samples of quail from the San Joaquin Experimental Range, Madera County, California.

The type of food consumed by these birds varies according to the season. They subsist primarily on seeds during the dry summer and early fall, but after the first rains the diet becomes predominantly green leafy material, as demonstrated by Glading, Biswell and Smith (1940) from the analysis of quail stomachs taken on the experimental range.

Data for the present study are based on the examination of 342 fecal samples procured from April 1941, through February 1942. The procedure followed has been described by Herman, Jankiewicz and Saarni. Intensity was computed by the number of oöcysts present in a single drop of surface film of the concentrated zinc sulfate centrifuged material (1–10, 1 - ; 11–50, 2 - ; 51–100, 3 - ; 101–500, 4 - ; 501–1000, 5 ; over 1000, 6). Tabulations were made by individual samples, no consideration being given to individual birds. In computing the data presented in section C of the graph, negatives were considered as 0, each 1 - infection as 1, etc. The average (points plotted) was obtained by dividing the sum of the infections for each month by the number of samples examined. Section A of the graph presents the actual inches of rainfall during the period of the study. Section B pictures the probable food consumption (for the period March 1941, through February 1942) interpolated from 1937 data of relation of rainfall to per cent of composition of food consumed (after Glading, Biswell and Smith, 1940).

Becker and Waters (1939) reported that alfalfa contains liberal amounts of the hypothetical materials for promoting coccidium growth as compared with grains,

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[2] Formerly Technical Advisor, Los Angeles Wildlife Disease Research Station.

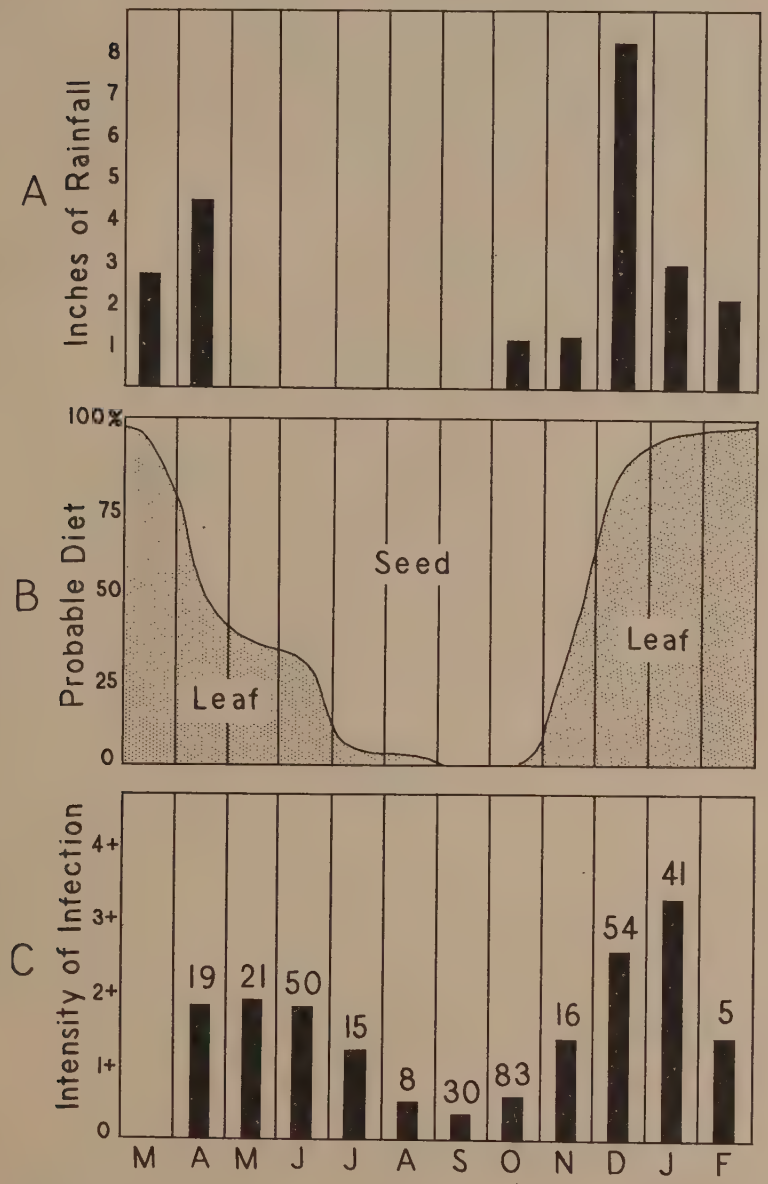

FIG. 1. Graphs indicating correlation of food habits with intensity of coccidian infection. A, inches of rainfall March 1941, through February 1942; B, probable food consumption (for period March 1941, through February 1942) interpolated from 1937 data of relation of rainfall to per cent of composition of food consumed (after Glading, Biswell and Smith, 1940); C, average intensity of coccidian infection, the figures above each bar indicating the number of fecal samples examined.

and we conjecture that other green foods may act similarly. The scope of this study has been limited by the relatively small number of samples examined and by its duration of only one year; also, very few samples were procured during some of the months. However, upon examination of the tabulated data as presented in the graphs, there seems to be a correlation between the consumption of green feed and the intensity of coccidian infection in quail living in a state of nature.

References

Becker, E. R. and Waters, P. C. 1939 Biological assay of feeding stuffs in a basal ration for coccidian-growth-promoting substance. III. Dried fish meal, alfalfa meal and white wheat flour. Iowa State Coll. J. Sc. **13**: 243–247.

Glading, B., Biswell, H. H. and Smith, C. F. 1940 Studies of the food of the California quail in 1937. J. Wildlife Management **4**: 128–144.

Herman, C. M. and Jankiewicz, H. A. 1942 Reducing coccidiosis in California valley quail during captivity. California Fish and Game **28**: 148–149.

Herman, C. M., Jankiewicz, H. A. and Saarni, R. W. 1942 Coccidiosis in California quail. Condor **44**: 168–171.

SOME OBSERVATIONS ON SPLEEN VOLUME IN DOMESTIC FOWLS IN THE COURSE OF *PLASMODIUM GALLINACEUM* STUDIES[1]

Paul F. Russell, Badri Nath Mohan and Persis Putnam

The lymphoid-macrophage system is a most important factor in the resistance of an animal to malaria. The spleen is a large element in this system and must be considered in any account of the physical or chemical bases of protozoan immunity. (For reviews and references see Taliaferro, 1929 and 1941; Culbertson, 1941; and Hewitt, 1940. Perla and Marmorston, 1935, discuss the human spleen in relation to resistance.)

This paper reports some observations on spleen volume in domestic fowls made in the course of studies on *Plasmodium gallinaceum* infections.

Spleen volume in nonmalarious fowls

In Table 1 are given data regarding spleen volume in 17 nonmalarious, and probably normal, domestic fowls. The fowls averaged about 10 weeks in age when purchased, but it was not possible to ascertain exact figures. It will be seen, in Table 1, that the average weight of the nonmalarious fowls was 634 grams and that the average spleen volume was 0.88 cc. This volume was obtained by noting the displacement of the spleen in a measuring cylinder. The spleen was carefully removed while the fowl was anaesthetized with chloroform.

Method of analysis

Obviously spleen volume is greater in heavier fowls, and it was necessary to find an expression of volume which would take into account the weight of the fowl. This was done by computing the correlation and fitting a regression line to the data for the 17 normal fowls given in Table 1. The correlation coefficient was found to be

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TABLE 1.—*Non-malarious untreated fowls: observed spleen volume and that computed from the regression of spleen volume on fowl weight**

Fowl serial number	Weight in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
		Observed	Expected	
255	724	1.2	1.08	+ 0.12
256	625	0.9	0.86	+ 0.04
262	862	2.0	1.38	+ 0.62
263	519	0.75	0.63	+ 0.12
264	663	0.75	0.95	− 0.20
275	560	0.6	0.72	− 0.12
276	553	0.5	0.71	− 0.21
277	725	0.8	1.08	− 0.28
278	555	0.5	0.71	− 0.21
279	606	1.2	0.82	+ 0.38
280	493	0.9	0.58	+ 0.32
298	577	0.75	0.76	− 0.01
299	630	1.5	0.87	+ 0.63
300	610	0.5	0.83	− 0.33
301	655	0.6	0.93	− 0.33
302	605	0.8	0.82	− 0.02
303	815	0.75	1.27	− 0.52
Total	10,777	15.00	15.00	0.00
Average per-fowl	634	0.88	0.88	0.00 ± 0.08

* Regression equation for computing theoretical values: Spleen volume in cc = 0.002165 (fowl weight in grams) − 0.490129.

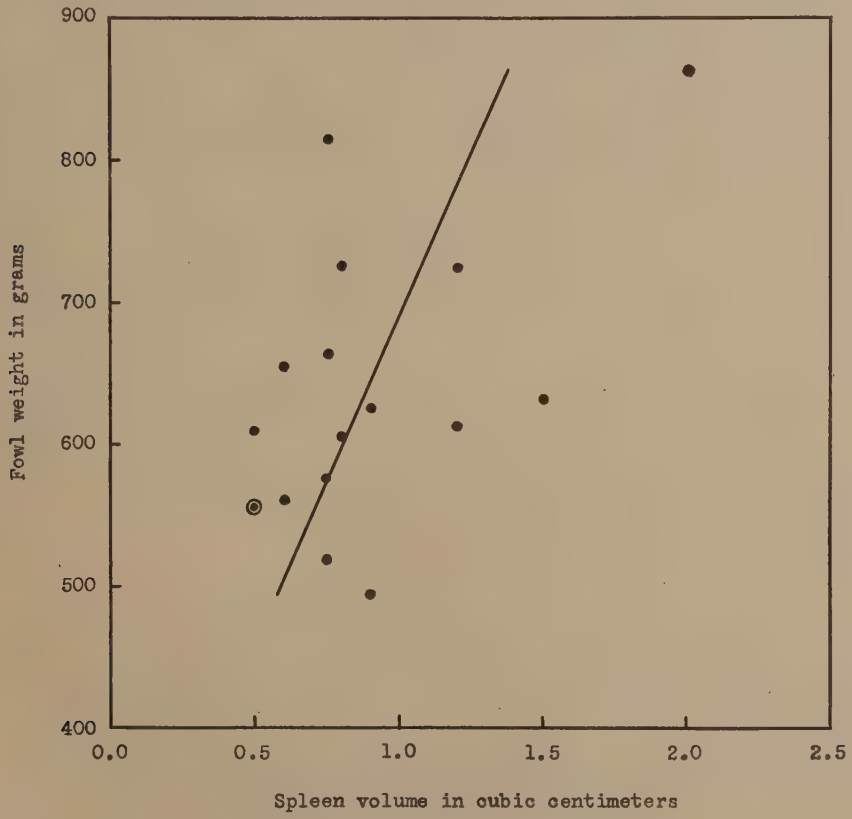

FIG. 1. Diagram showing the regression of spleen volume on fowl weight for a group of 17 normal birds.

+ 0.500, and the regression of spleen volume on weight is expressed by the following equation:

Spleen volume in cc = 0.002165 (fowl weight in gms) − 0.490129. The correlation diagram with the fitted line for 17 normal fowls is shown in Fig. 1.

It is apparent that the observed values of weight and volume deviate considerably from the fitted line, particularly those for the heavier birds. The amount of deviation may be expressed in the form of a standard error[2] which will be smaller than one based on deviations from the average of observed spleen volumes, since the increase in volume per increase in weight has been taken into consideration. In Table 1 the sum of the deviations shown is zero with a standard error of ± 0.08 cc.

If the equation may be considered an adequate expression of the normal relationship between spleen volume and body weight it may be used to compute the expected spleen volumes for the fowls in each treated group. These expected values may then be compared with the observed spleen volumes following treatment. Each expected value will correspond to a point on the line shown in Fig. 1, and the amount and direction of observed from computed values may be used as a measure of the effect of treatment.

Fisher, 1938, gives a t test which may be used for determining the statistical significance of the differences between expected and observed values. The tables which follow contain the individual differences and their mean with standard error[3] to which the test was applied. While the standard error does not take into account all possible variation of normal versus experimental spleen volumes, the differences in most instances are so great that the test is satisfactory for the purpose of this paper.

EFFECT OF INJECTIONS OF SHEEP SERUM

In Table 2 are included data for 17 fowls which received injections of normal sheep serum intravenously, subcutaneously, or intraperitoneally. Fowls 247 and 248 received intravenous injections of 1 cc sheep serum on March 31, April 7, and April 14, 1941. The spleens were removed on April 15. Fowls 259 to 261 received 0.25 cc sheep serum subcutaneously over the breast muscle daily from April 15 to 21, 1941. Spleens were removed on May 3. Fowls 281 to 286 received similar injections every second day from May 3 to 15, 1941. Spleens were removed on May 17.

The average weight of these fowls was 636 grams and the average expected spleen volume was 0.89 cc, about the same as in the untreated group. But the average observed spleen volume was 1.68 cc, nearly double that expected. The difference of + 0.79 ± 0.12 cc is significant. Evidently there was an increase in splenic volume following these injections.

EFFECT OF VACCINATION WITH INACTIVATED SPOROZOITES

In Table 3 are given data for seven fowls which received sporozoite vaccination. Each fowl received sporozoites from 200 mosquitoes injected intravenously in five equal doses between December 23 and January 20. In the case of fowls D 43, 45, and 54, salivary glands were dissected in saline and exposed to ultraviolet radiation for 30 minutes before injection. In fowls 47, 49, 51 and 52, thoraces from infective

[2] Standard error = $\left(\frac{\Sigma(\text{differences observed vs. expected volumes})^2}{n(n-2)} \right)^{\frac{1}{2}}$ in which n is equal to the number of fowls in the group.

[3] Standard error computed as above with $n(n-1)$ as the denominator.

TABLE 2.—*Spleen volume of non-malarious fowls after injections of sheep serum*

Fowl serial number	Injections			Weight in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
	No.	Amount total cc	Route		Observed	Expected	
247	3	3.0	i/v	723	2.5	1.08	+ 1.42
248	3	3.0	i/v	751	1.75	1.14	+ 0.61
259	7	1.75	s/c	504	1.5	0.60	+ 0.90
260	7	1.75	s/c	575	1.9	0.75	+ 1.15
261	7	1.75	s/c	670	1.5	0.96	+ 0.54
265	7	7.0	i/p	587	1.7	0.78	+ 0.92
266	7	7.0	i/p	449	1.5	0.48	+ 1.02
267	7	7.0	i/p	583	2.25	0.77	+ 1.48
268	7	7.0	i/p	550	1.5	0.70	+ 0.80
269	7	7.0	i/p	773	1.9	1.18	+ 0.72
270	7	7.0	i/p	676	1.6	0.97	+ 0.63
281	7	7.0	i/p	742	2.9	1.12	+ 1.78
282	7	7.0	i/p	570	1.0	0.74	+ 0.26
283	7	7.0	i/p	729	1.2	1.09	+ 0.11
284	7	7.0	i/p	682	1.75	0.99	+ 0.76
285	7	7.0	i/p	612	1.0	0.83	+ 0.17
286	7	7.0	i/p	643	1.1	0.90	+ 0.20
		Totals		10819	28.55	15.08	+ 13.47
		Average per fowl		636	1.68	0.89	+ 0.79 ± 0.12

mosquitoes were ground and dried and a saline suspension was injected. (For details regarding technique and significance of these procedures see Mulligan et al, 1941; Russell et al, 1942; Russell and Mohan, 1942.) The fowls were sacrificed on the day after receiving the last injection of vaccine.

The average weight of these seven fowls was 631 grams, and the expected average spleen volume was 0.88 cc, the same as that observed in normal fowls in Table 1. The average observed value, however, was 1.53 cc and the difference between expected and observed values was + 0.65 ± 0.18 cc and significant.

It should be noted that the observed spleen volumes in Tables 2 and 3 are all in excess of the expected values. This is a further indication that the spleens of these birds were abnormally large. If the deviations were distributed both below and above the expected values one could not be so sure of this.

EFFECT OF MALARIA

In Tables 4 and 5 data are given for spleens removed from fowls suffering from chronic malaria. The eight fowls reported in Table 4 were infected by blood inocu-

TABLE 3.—*Spleen volume of non-malarious fowls after vaccinations with inactivated sporozoites*

Fowl serial number	Method of inactivating sporozoites	Weight of fowl in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
			Observed	Expected	
D 43	u/v light	649	1.0	0.91	+ 0.09
D 45	u/v light	620	2.2	0.85	+ 1.35
D 54	u/v light	663	2.2	0.95	+ 1.25
Totals		1932	5.4	2.71	+ 2.69
Average per fowl		644	1.80	0.90	+ 0.90
D 47	Drying and grinding	654	1.2	0.93	+ 0.27
D 49	Drying and grinding	712	1.8	1.05	+ 0.75
D 51	Drying and grinding	445	0.8	0.47	+ 0.33
D 52	Drying and grinding	675	1.5	0.97	+ 0.53
Totals		2486	5.3	3.42	+ 1.88
Average per fowl		622	1.33	0.86	+ 0.47
Grand totals		4418	10.7	6.13	+ 4.57
Average per fowl		631	1.53	0.88	+ 0.65 ± 0.18

TABLE 4.—*Spleen volume of untreated chronic malarious fowls infected by blood inoculations*

Fowl serial number	Duration of infection days	Peak of infection	Weight of fowl in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
				Observed	Expected	
223	38	+++++	609	2.8	0.83	+ 1.97
226	38	+++++	591	1.5	0.79	+ 0.71
227	38	+++++	760	4.1	1.16	+ 2.94
228	38	+++++	657	2.8	0.93	+ 1.87
230	38	+++++	639	3.0	0.89	+ 2.11
254	114	++	854	1.4	1.36	+ 0.04
258	38	++	625	1.8	0.86	+ 0.94
305	33	+	656	1.8	0.93	+ 0.87
Totals	375	....	5391	19.2	7.75	+ 11.45
Averages	47	....	674	2.40	0.97	+ 1.43 ± 0.33

NOTES: The plus signs follow Beckman's (1941) method of classification. As used by us they have approximately the following meaning:

\+ Less than 1 per cent of cells infected
++ One to 4 per cent of cells infected
+++ Five to 8 per cent of cells infected
++++ Nine to 12 per cent of cells infected
+++++ More than 12 per cent of cells infected

lation and the 26 in Table 5 by mosquito-borne infection. The average weight of these 34 fowls was 657 grams when sacrificed, a little more than that of newly purchased birds since they had been well fed in the laboratory for several weeks. The observed average spleen volume of the 34 fowls was 2.28 cc. The expected average spleen volume was 0.93 cc, giving a difference of + 1.35 ± 0.16 cc, which is highly significant.

The difference in spleen volume between fowls infected by needle and by mosquito inoculation is not significant, either when comparison is made of the average observed or expected values. The mean duration of infection in the first group was 47 days, and in the second 39 days, from date of inoculation to that of removal of spleen.

TABLE 5.—*Spleen volume of untreated chronic malarious fowls infected by mosquitoes*

Fowl serial number	Duration of infection in days	Peak of infection	Weight of fowl in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
				Observed	Expected	
195	80	++	899	1.2	1.46	− 0.26
197	78	+	892	1.9	1.44	+ 0.46
219	55	+	696	1.5	1.02	+ 0.48
220	55	+	658	1.8	0.93	+ 0.87
320	24	++++	428	2.2	0.44	+ 1.76
321	23	++++	654	2.8	0.93	+ 1.87
323	23	+++++	1007	3.8	1.69	+ 2.11
325	23	+++++	564	2.7	0.73	+ 1.97
326	23	++++	507	1.8	0.61	+ 1.19
327	23	+++	686	4.0	1.00	+ 3.00
328	23	+++++	433	2.7	0.45	+ 2.25
395	55	+++++	486	1.0	0.56	+ 0.44
398	54	+++++	484	1.2	0.56	+ 0.64
400	73	+++++	671	1.8	0.96	+ 0.84
413	41	++++	629	2.0	0.87	+ 1.13
414	41	++++	657	1.5	0.93	+ 0.57
415	41	+++++	612	4.0	0.83	+ 3.17
416	40	++++	588	2.0	0.78	+ 1.22
418	30	++++	597	1.4	0.80	+ 0.60
419	40	+++++	461	1.8	0.51	+ 1.29
420	40	++++	702	1.9	1.03	+ 0.87
421	39	++++	453	0.9	0.49	+ 0.41
423	39	+++	736	3.0	1.10	+ 1.90
A 94	21	++	959	5.0	1.59	+ 3.41
A 97	21	+++	743	2.4	1.12	+ 1.28
A 98	21	++	739	2.0	1.11	+ 0.89
Total	1026	....	16941	58.3	23.94	+ 34.36
Average	39	....	652	2.24	0.92	+ 1.32 ± 0.18

That the spleen volume per fowl weight decreases as the infection becomes more chronic in these fowls is seen in the following arrangement of data from Tables 4 and 5.

Duration of infection in days	Number of fowls	Average weight in gms	Average spleen volume in cc		Difference observed vs. expected
			Observed	Expected	
20–29	10	672	2.94	0.97	+ 1.97 ± 0.25
30–39	10	632	2.31	0.88	+ 1.43 ± 0.26
40–49	6	608	2.20	0.83	+ 1.38 ± 0.37
50 or more	8	705	1.48	1.04	+ 0.44 ± 0.14

The excess of observed over expected spleen volume decreased as the duration of infection increased. The amount of the excess was significant for each group.

EFFECT OF SPOROZOITE VACCINATION WITH SUBSEQUENT MALARIA INFECTION

In Tables 6 and 7 are reported data regarding spleen volume in 14 fowls that

TABLE 6.—*Spleen volume of vaccinated chronic malarious fowls infected by blood inoculation*

Fowl serial number	Duration of infection in days	Peak of infection	Weight of fowl in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
				Observed	Expected	
203	37	+++++	705	2.0	1.04	+ 0.96
204	37	++++	756	4.0	1.15	+ 2.85
208	37	+++++	710	2.75	1.05	+ 1.70
210	37	++++	1035	3.0	1.75	+ 1.25
211	37	+++++	750	3.9	1.13	+ 2.77
Totals	185	....	3956	15.65	6.12	+ 9.53
Average	37	....	791	3.13	1.22	+ 1.91 ± 0.39

TABLE 7.—*Spleen volume of vaccinated chronic malarious fowls infected by mosquitoes*

Fowl serial number	Duration of infection in days	Peak of infection	Weight of fowl in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
				Observed	Expected	
165	83	+++++	1317	2.0	2.36	− 0.36
171	83	+++++	1036	2.0	1.75	+ 0.25
176	53	+++	947	2.1	1.56	+ 0.54
177	53	+++	768	1.75	1.17	+ 0.58
185	61	+++	869	2.2	1.39	+ 0.81
A 7	24	+	698	3.4	1.02	+ 2.38
A 11	24	+	734	2.2	1.10	+ 1.10
A 13	24	+	579	0.9	0.76	+ 0.14
A 22	24	+	679	3.1	0.98	+ 2.12
Totals	429	....	7627	19.65	12.09	+ 7.56
Averages	48	....	847	2.18	1.34	+ 0.84 ± 0.30

were given sporozoite vaccination and subsequently infected with malaria. Those included in Table 6 were infected by blood inoculation, and all had heavy infections. Those shown in Table 7 were infected by mosquito-borne sporozoites, and all had light infections. (For description of vaccination technique and other details regarding fowls included in Table 6 see Russell et al, 1942; for fowls 165 to 185 in Table 7 see Mulligan et al, 1941; for fowls A 7 to A 22 see Russell and Mohan, 1942.)

In the trophozoite inoculated group (Table 6) the difference between average observed and expected spleen volumes was + 1.91 ± 0.39 cc. In the sporozoite

infected group (Table 7) the difference was + 0.84 ± 0.30 cc. In both groups the observed spleens were significantly larger than they presumably would have been if normal.

EFFECT OF SERUM INJECTIONS WITH SUBSEQUENT MALARIA INFECTION

In Table 8 are data regarding four fowls which were infected after receiving

TABLE 8.—*Spleen volume of chronic malarious fowls, treated with serum, infected by mosquitoes*

Fowl serial number	Duration of infection in days	Peak of infection	Weight of fowls in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
				Observed	Expected	
359	61	++++	504	1.1	0.60	+ 0.50
360	61	+	1040	2.5	1.76	+ 0.74
368	61	+++++	431	0.9	0.44	+ 0.46
A 91	21	++	586	3.8	0.78	+ 3.02
Totals	204	....	2561	8.3	3.58	+ 4.72
Average	51	....	640.3	2.08	0.90	+ 1.18 ± 0.62

serum injections. Fowls 359, 360, and 368 each received 1 cc of sheep serum daily from June 23 to 30, 1941, intraperitoneally. They were bitten by two infective mosquitoes on July 1. The infection ran for 61 days before the spleens were removed. Fowls 359 and 368 had severe infections, but in fowl 360 the infection was mild. Fowl A91 received daily injections of 1 cc of chronic fowl serum intraperitoneally from September 15 to 20. It was bitten by two infective mosquitoes on September 17.

The excess in observed spleen volume over that expected was small among the fowls in this group, with the exception of fowl A 91. The long duration of the disease in the others may have been the cause of this.

EFFECT OF COMBINED SPOROZOITE VACCINATION AND SERUM : INJECTIONS WITH SUBSEQUENT MALARIA INFECTION

In Table 9 data for 17 fowls are shown, each of which had both sporozoite vac-

TABLE 9.—*Spleen volume of chronic malarious fowls, vaccinated and treated with serum, infected by mosquitoes*

Fowl serial number	Duration of infection in days	Peak of infection	Weight of fowl in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
				Observed	Expected	
233	23	+++++	875	3.7	1.40	+ 2.30
234	23	+++++	898	5.0	1.45	+ 3.55
235	23	+++++	912	5.5	1.48	+ 4.02
236	22	+++++	660	4.3	0.94	+ 3.36
237	22	++++	924	3.5	1.51	+ 1.99
238	22	+++++	719	3.2	1.07	+ 2.13
239	30	+++++	679	3.0	0.98	+ 2.02
241	22	+++++	691	3.7	1.01	+ 2.69
272	22	+++++	677	3.2	0.98	+ 2.22
357	60	+++++	747	3.7	1.13	+ 2.57
393	42	+++++	692	3.1	1.01	+ 2.09
401	40	++++	914	3.0	1.49	+ 1.51
A 46	21	+	890	3.5	1.44	+ 2.06
A 48	21	+	758	3.6	1.15	+ 2.45
A 51	21	+	793	3.0	1.23	+ 1.77
A 53	21	+	981	4.8	1.63	+ 3.17
A 60	21	+	1021	4.5	1.72	+ 2.78
Totals	456	....	13831	64.3	21.62	+ 42.68
Averages	27	....	814	3.78	1.27	+ 2.51 ± 0.16

cination and serum injection (see Russell and Mohan, 1942). Fowls 233 to 272 were each vaccinated five times between April 17 and May 12. They also received 1 cc of sheep serum intraperitoneally daily from May 10 to 16. They were infected by two mosquitoes on May 19. Fowls 357 to 401 were vaccinated six times between June 22 and July 14, 1941, and each received 1 cc chronic fowl serum intraperitoneally daily from July 14 to 18. They were infected by two mosquitoes on July 20. Fowls A 46 to 60 were vaccinated five times between August 29 and September 13. They also received 1 cc chronic fowl serum daily by intraperitoneal injection from September 15 to 20. They were infected by two mosquitoes on September 17.

The excess of observed over expected spleen volume was greatest in this group of fowls. The average difference was $+2.51 \pm 0.16$ cc and is highly significant.

DISCUSSION

Table 10 summarizes the results of the observations made on these 110 fowls.

TABLE 10.—*Results of experiments designed to show the effect of various types of treatment upon the average spleen volume of fowls*

Table number	Description of fowl group	Number of fowls	Average weight in grams	Average spleen volume in cubic centimeters		Difference observed vs. expected with standard error
				Observed	Expected	
1	Nonmalarious, untreated	17	634	0.88	0.88	0.00 ± 0.08
2	Nonmalarious, serum treated	17	636	1.68	0.89	0.79 ± 0.12
3	Nonmalarious, vaccinated	7	631	1.53	0.88	0.65 ± 0.18
4	Chronic-malarious, blood inoculated	8	674	2.40	0.97	1.43 ± 0.11
5	Chronic-malarious, mosquito infected	26	652	2.24	0.92	1.32 ± 0.18
6	Chronic-malarious, vaccinated, blood-inoculated	5	791	3.13	1.22	1.91 ± 0.39
7	Chronic-malarious, vaccinated, mosquito infected	9	847	2.18	1.34	0.84 ± 0.30
8	Chronic-malarious, serum treated, mosquito infected	4	640	2.08	0.90	1.18 ± 0.62
9	Chronic-malarious, vaccinated and serum treated, mosquito infected	17	814	3.78	1.27	2.51 ± 0.16

The spleen in 17 normal fowls about 10 weeks in age averaged 0.88 cc in volume. It was possible to compute the regression of spleen volume on body weight for these normal fowls, by means of which the expected spleen volume in terms of weight of fowl could be computed for each bird in the experimental groups. This was done, and comparisons were made between observed and theoretical spleen volumes so that the effect of treatment could be evaluated. The following observations may be made:

Vaccination with inactivated sporozoites, whether the vaccine is prepared by exposure to ultraviolet radiation or by grinding and drying, increases the volume of the spleen in nonmalarious fowls.

The injection of serum, whether from normal sheep or from fowls chronically infected with the homologous plasmodium, causes the spleen to increase in volume. Sections from such spleens showed chiefly an increase in lymphoid tissue with somewhat enlarged sinuses engorged with lymphoid cells.

Manwell and Goldstein, 1940, observed this effect of serum upon the spleens of nonmalarious canaries. They found that injections of normal serum or even of physiological saline produced a splenic enlargement comparable to that produced by immune serum.

The spleen volume is increased by malaria infection (Tables 4 and 5) and there is no significant difference between blood-inoculated and mosquito-infected groups. Such enlargement has, of course, long been known to occur. It was interesting that the volume decreased as the chronic infection progressed.

The largest increase in volume was seen in the group of 17 chronically malarious fowls which had had both vaccination and serum treatment in addition to mosquito-borne malaria infection (Table 9).

SUMMARY

This paper presents data showing the spleen volume of normal fowls and the increased volume caused by chronic malaria, by serum treatment, by sporozoite vaccination, and by chronic malaria following sporozoite vaccination with and without serum treatment.

REFERENCES

1. BECKMAN, H. 1941 An experimentally derived method for determining the degree of infection in avian malaria. Am. J. Trop. Med. **21**: 151–157.
2. CULBERTSON, J. T. 1941 Immunity Against Animal Parasites. Columbia Univ. Press, New York. 241 pp.
3. FISHER, R. A. 1938 Statistical Methods for Research Workers. 7th ed. Oliver and Boyd, London.
4. HEWITT, REDGINAL 1940 Bird Malaria. Am. J. Hyg. Monogr. Ser. 15.
5. MANWELL, R. D. AND GOLDSTEIN, F. 1940 Passive immunity in avian malaria. J. Exper. Med. **71**: 409–422.
6. MULLIGAN, H. W., RUSSELL, P. F. AND MOHAN, B. N. 1941 Active immunization of fowls against *Plasmodium gallinaceum* by injections of killed homologous sporozoites. J. Mal. Inst. India **4**: 25–34.
7. PERLA, D. AND MARMORSTON, J. 1935 The Spleen and Resistance. Williams & Wilkins Co., Baltimore.
8. RUSSELL, P. F. AND MOHAN, B. N. 1942 The immunization of fowls against mosquito-borne *Plasmodium gallinaceum* by injections of serum and of inactivated homologous sporozoites. J. Exper. Med. **76**: 477–495.
9. RUSSELL, P. F., MULLIGAN, H. W. AND MOHAN, B. N. 1942 Active immunization of fowls against sporozoites but not trophozoites of *P. gallinaceum* by injections of homologous sporozoites. J. Mal. Inst. India **4**: 311–319.
10. TALIAFERRO, W. H. 1929 The Immunology of Parasitic Infections. Century Co., New York.
11. TALIAFERRO, W. H. 1941 The immunology of the parasitic protozoa. Chap. XVIII, in "Protozoa in Biological Research," edited by G. N. Calkins and F. M. Summers. Columbia Univ. Press, New York.

OBSERVATIONS ON THE SEGMENTAL ANATOMY OF THE TAPEWORM, *MESOCESTOIDES VARIABILIS* MUELLER, 1928, FROM THE OPOSSUM

ELON E. BYRD
Zoology Department, University of Georgia
AND
JAMES W. WARD
Department of Zoology and Entomology, Mississippi State College

Recently one of us (J.W.W.) obtained several incomplete specimens of a tapeworm from the small intestine of the opossum, *Didelphis virginiana* Kerr, from Mississippi. Unfortunately only incomplete strobilae were obtained and in no case was a scolex recovered. Hence no measurements on the total length of single specimens can be made nor can we determine the total number of proglottides in the strobila of the individual. However a sufficient number of both mature and gravid proglottides was obtained for making whole mounts and sections. A study of these segments from the same chain and from similar chains in the collection has convinced us all of our material belongs to the same species of tapeworm.

Although our material came from the opossum, the size of the mature and gravid proglottides, the nature and arrangement of the musculature within the segment, the presence of rather large calcareous corpuscles and the nature of the egg-capsule of the fully gravid segment definitely determine the species to be identical with *Mesocestoides variabilis* Mueller, 1928.

Since no complete strobilae were obtained we are permitted only to give a description of the anatomy of the segment. Such a detailed description seems to be timely since in our opinion no paper familiar to us on the group of tapeworms here considered adequately describes the connections made by the various ducts that compose the reproductive system. Therefore in the present paper we propose to give a somewhat extended description of the internal anatomy of the species and to present our observations on the dorso-ventral axis of the strobila as worked out by us from cross, sagittal and frontal sections of both the mature and gravid proglottides.

THE MATURE PROGLOTTID

(Figs. 1–4)

The mature proglottid, in which all essential anatomical features are well enough defined to be recognizable, has the following dimensions: length from 200 to 250 μ; width from 1.25 and 1.50 mm; thickness from 700 to 830 μ. The segments, therefore, are strongly ovoid in cross section, being only slightly dorso-ventrally flattened. The margins of the segments are irregular in profile and the strobila is deeply indented at the union of the segments, giving the chain a marked serrated appearance (Figs. 1 and 4). The surface of the segment is covered by a relatively thick cuticle which becomes thinner along the posterior margin of the indented boundary and in the area of the genital pore. Just underneath the cuticle lies a more or less homogeneous matrix of cells from which the cuticle arises. This is followed by a rather

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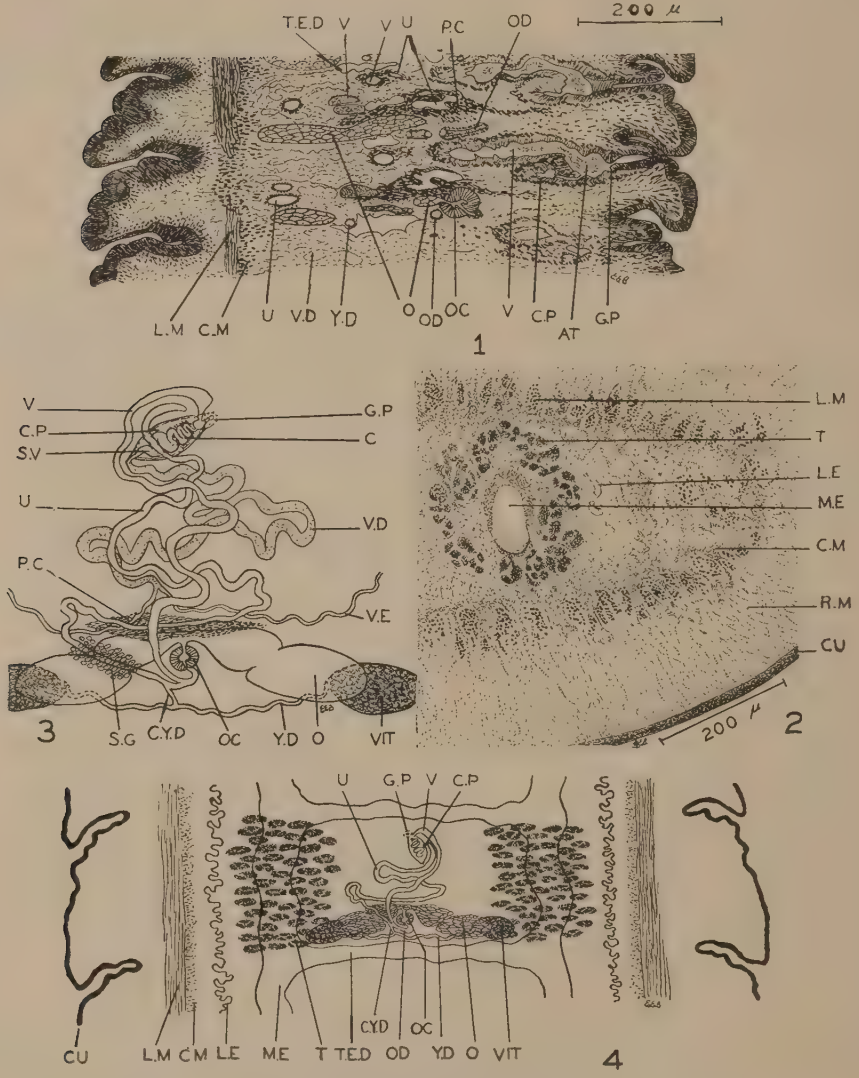
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200 μ
L.M C.M U V.D Y.D O OD OC V C.P AT G.P
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V C.P S.V U P.C G.P C V.D V.E
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S.G C.Y.D OC Y.D O VIT
L.M T L.E M.E C.M R.M CU
200 μ
2
U G.P V C.P
CU L.M C.M L.E ME T T.E.D OD C.Y.D OC Y.D O VIT
4

Mesocestoides variabilis Mueller, 1928

FIG. 1. Slightly diagonal, sagittal section (drawn with aid of camera lucida) of mature segment in the region of the genital pore, showing the dorsal position of the genital pore and the ventral position of the ovary.

FIG. 2. Cross section (drawn with aid of camera lucida) of mature segment, showing position and arrangement of muscle layers, position of lateral and median excretory canals on one side and arrangement of the testes about the median excretory canal.

FIG. 3. Diagrammatic sketch, showing the general arrangement of the reproductive system within a single mature proglottid. Ventral view.

FIG. 4. Camera lucida outline of a single mature segment with diagrammatic sketch of both male and female organs, showing their relationship to each other and the positions of the excretory canals. Ventral view.

light band of circular and radial muscle fibers. The radial fibers of this area anastomose with fibers from another band of radial muscles which is arranged with another band of circular muscles in the area of the longitudinal muscles. The conspicuous band of longitudinal fibers, together with the circular and radial bands, make up the principal muscular area of the body (Figs. 1, 2 and 4). This muscular area forms a complete band of muscles surrounding the principal organs of the reproductive and excretory systems. In cross and sagittal sections the band of muscles lies from one-third to one-fourth the diameter of the section from the surface. The longitudinal muscles (L.M) are arranged in definite bundles, each bundle being composed of several strands of fibers and separated from adjacent bundles by the circular and radial fibers. Just mesal to the bundles of the longitudinal fibers lies the most dense concentration of circular (C.M) and radial fibers (Fig. 2, R.M). All important organs except the terminal portions of the genital ducts and the genital pore lie within the area inclosed by the muscular layers. Thus the segment is separated into inner, medullary, and outer, cortical, areas by the bands of muscles.

The male reproductive system. The testes number between 45 and 55 follicles in each side of the segment. They are arranged in irregular rows about the main excretory ducts, the rows completely encircling the main excretory canal throughout the entire length of the canal within the segment. The follicles of the testes (Figs. 2 and 4, T) are round to ovoid in outline and measure up to 60 μ in diameter. They appear to be masses of cell accumulations which are confined within a definite membrane. Although individual sperm ducts were not seen to arise from each testicular body there is evidence in our sections to indicate their presence. A single, rather large sperm duct, the vas efferens (Fig. 3, V.E), arises from each group of the testes and passes postero-mesiad from the mid-region of the testicular group to a point just anterior to the ovary. At this point the two vasa efferentia become fused into the formation of a somewhat dilated vas deferens. The vas deferens (Figs. 1 and 3, V.D) becomes convoluted and forms antero-posterior as well as extensive transverse loops in ascending through the medullary portion of the segment to enter the cirrus sac. On entering the cirrus sac the vas deferens becomes more dilated and is thrown into three or four corkscrew-like coils, forming the seminal vesicle (Fig. 3, S.V). Following this the duct develops a thickened, muscular wall, the cirrus, and passes to the opening into the atrium. The muscular portion of the tube is surrounded by a dense mass of deeply staining gland cells. The cirrus is eversible and is seen frequently to protrude through the genital pore for a distance of from 60 to 100 μ. The cirrus pouch (Figs. 1, 3 and 4, C.P) is a thin-walled, weakly muscular structure surrounding the seminal vesicle and cirrus. It measures from 30 to 70 μ in width by 60 to 150 μ in length. The pouch may lie ventro-lateral, ventral, postero- or antero-ventral to the genital pore, however, it is more commonly found lying in an antero-ventral position, especially in the fully gravid proglottid.

The genital pore. The genital pore (Figs. 1, 3 and 4, G.P) is located in the midline of the dorsal surface of the segment. It opens to the outside through a pore situated in the floor of a depression in the cuticle in the area of the pore. Internally the pore leads into a flask-shaped atrium. The male duct opens into the atrium from the postero-ventral aspect while the female duct connects with it from the antero-ventral position.

The female reproductive system. The bilaterally lobed ovary (Figs. 1, 3 and 4, O) is situated ventrally, close to the posterior margin of the segment. The two

lobes arch antero-dorso-mesally from a point just ventro-mesally to the area of the testes, in the ventral aspect of the medullary portion of the segment, and are joined mesally by an isthmus of cells. Hence the main body of the ovary lies ventrally to the plane of the longitudinal and transverse excretory canals while the isthmus generally lies dorsal to that plane. The ovary, therefore, due to its arched position, roughly resembles a leaning, inverted U. Each lobe of the ovary is indistinctly separated into two lobes by an indentation near the middle of the lobe (Figs. 3 and 4, O) and is flattened antero-posteriorly (Fig. 1, O). The entire body of the ovary is more or less a loose mass of elongated, ovoid-shaped cells, surrounded by a very delicate and inconspicuous membrane. Each lobe measures from 50 to 60 μ in length, 150 to 180 μ in width and 150 to 230 μ in depth. The distance between the lobes is very variable.

The oviduct arises from the dorsal aspect of that part of the ovarian isthmus which lies dorsal to the plane of the excretory canals. It passes posteriorly from the point of its origin to near the caudal margin of the segment, then turns anteriorly. Almost at once the oviduct develops the conspicuous oöcapt (Figs. 1, 3 and 4, OC). Beyond this the oviduct continues posteriorly to near the caudal boundary of the segment and then swings ventrally and anteriorly and passes anteriorly to about the level of the isthmus before giving rise to the uterus and vagina. From the point of its origin the uterus passes antero-laterally and is soon joined by the common yolk duct (Figs. 3 and 4, C.Y.D). A short distance from this fusion the uterus becomes surrounded by a mass of unicellular gland cells, the shell gland (Fig. 3, S.G). Following this the uterus curves mesally and passes across the segment to the opposite side, passing close in front of the ovary. From the opposite side of the segment the uterus begins an irregular series of transverse loops, ascending through the medullary portion of the segment to a level slightly in advance of the genital pore, where it ends blindly. In making the first transverse swing across the segment the uterus is completely embedded in a dense mass of deeply stained, spindle-shaped cells, the paruterine cells (Figs. 1 and 3, P.C). In segments in this stage of development there are few or no ova to be found within the uterus.

Throughout its entire length the vagina is a slightly convoluted tube (Figs. 1, 3 and 4, V). From its origin to the level of the genital pore the vagina lies ventral to the uterus, but passes around the uterus at the level of the genital pore and arches over the cirrus pouch to enter the atrium from above. As the vagina leaves the ventral position its wall becomes thickened and more muscular and the lumen greatly dilated (Figs. 1, 3 and 4, V). The dilated part of the vagina becomes filled with spermatozoa early in its development and, as Chandler (1942) suggests, probably serves as a receptaculum seminis.

The vitelline glands are composed of two compact lobes, one lobe of which lies on either side of the midline, in the medullary portion of the segment (Figs. 3 and 4, VIT). The lobes occupy a dorsal position within the segment and lie mostly lateral and posterior to the ovary. Each lobe is only about one-half the size of an ovarian lobe. From each lobe of the vitelline glands arises a very delicate yolk duct (Figs. 3 and 4, Y.D) and this passes mesiad, close beside the antero-mesal aspect of the transverse excretory canal, to near the midline of the segment where it joins with its fellow to form a short, common yolk duct. The common yolk duct merges with the uterus a short distance from the origin of the latter.

The excretory system. The excretory system of the mature segment consists of two pairs of longitudinal canals, one pair of which is connected by transverse canals. Both pairs of the canals lie in the medullary portion of the segment and are located on the same medio-frontal plane (Fig. 2). The more lateral pair of the canals consists of two very small, greatly convoluted canals, one on either side of the segment, between the area of the testes and the muscular area (Figs. 2 and 4, L.E). The more median pair, the main excretory canals, consists of two much larger canals. These are only gently undulated throughout their course and lie in the area of the testes, completely surrounded by the testes (Figs. 2 and 4, M.E). The main excretory canals are connected one with the other by the transverse canals. These lie in the area of the junction between adjacent segments (Figs. 1 and 4, T.E.D). Hence a single transverse canal serves the posterior portion of one segment and the anterior portion of the next.

THE GRAVID PROGLOTTID

(Figs. 5–9)

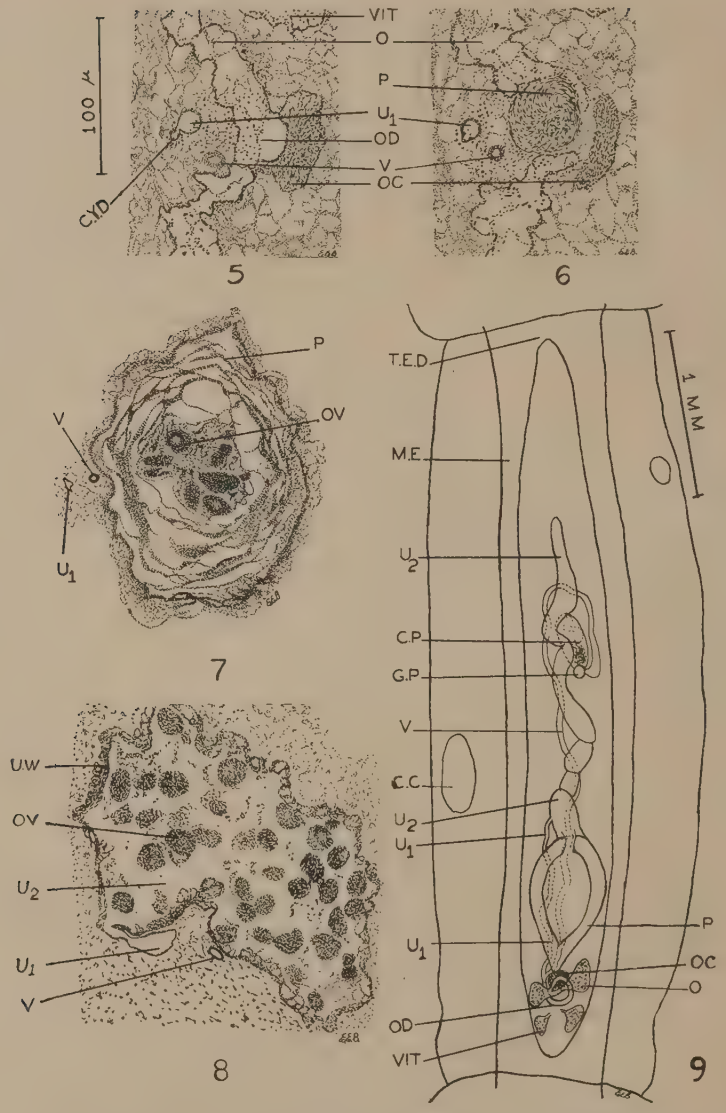
As the segment becomes gravid (gravid in the sense that only the vestige of the female organs and genital ducts can be made out and most of the ova have been crowded into the egg-capsule, paruterine organ) it gradually changes its shape from the condition of the mature segment to, first, a squarish shape and, then, to a greatly elongated one. The segment is decidedly dorso-ventrally flattened. The most gravid segment in the present collection has the following dimensions: length 3.85 mm; width at anterior end 1.13 mm, at genital pore 1.56 mm and at posterior end 1.35 mm.

In the mature segment an occasional calcareous corpuscle is present (in a chain of 34 segments which was sectioned only a single calcareous corpuscle appeared). In proportion to the size of the mature proglottid the calcareous corpuscle is very large, crowding the tissues and organs of the segment to such an extent that the entire segment is distorted. As the segment ripens the calcareous corpuscles become more numerous so that in the fully gravid segment as many as four or five rather large corpuscles may be present. These bodies are not found in every segment and there are usually no more than two to a segment. The calcareous corpuscle generally lies in the cortical portion of the segment, laterally to the excretory canals (Fig. 9, C.C).

The genital pore (Fig. 9, G.P) is dorsal in position and lies in the midline at a level approximating one-third the length of the segment from the anterior end. It measures about 70 μ in diameter.

The cirrus pouch and proximal portion of the vas deferens still persists in the fully gravid segment. The cirrus pouch lies in an antero-ventral position in relation to the genital pore and measures approximately 140 μ in length. Internally the cirrus and seminal vesicle can be made out in sectioned material. The proximal portion of the vas deferens can be traced posteriorly from the cirrus pouch for only a very short distance. In neither the whole mount nor the sectioned material is it possible for us to find any trace of the testes in the fully gravid proglottid.

In the whole mount the uterus and egg-capsule, paruterine organ, are the most conspicuous features within the proglottid. The egg-capsule (Figs. 7 and 9, P) consists of a thick-walled, blind evagination from the uterus, the lumen of which is filled with ova. It measures up to 700 μ in length by 400 μ in greatest width and may lie as close as 600 μ to the posterior margin of the segment. Anterior to the



Mesocestoides variabilis Mueller, 1928

FIG. 5. Cross section (drawn with aid of camera lucida) of gravid segment in region of isthmus of the ovary, showing origin of oviduct, the position of the ovarian lobes, the oöcapt, the vitellaria and the union of the common yolk duct with the uterus. The vitellaria and oöcapt lie dorsal to the median frontal section.

FIG. 6. Same as Fig. 5 except more anterior, at posterior end of paruterine appendage. Part of oöcapt can be seen within the section. Drawn with aid of camera lucida to the same scale as Fig. 5.

FIG. 7. Cross section of paruterine organ in gravid segment. Note vagina (V) on ventral aspect of paruterine organ, between it and the thin-walled, delicate uterus (U_1). Drawn with aid of camera lucida to the same scale as Fig. 5.

egg-capsule the uterus (Figs. 8 and 9, U_2) follows a slightly irregular course along the midline to a level approximately 1.00 mm from the anterior end of the segment, where it ends blindly. The uterus, therefore, terminates at a level in advance of the genital pore. In the gravid segment that part of the uterus anterior to the egg-capsule contains relatively few ova except in the area immediately adjacent to the egg-capsule. Throughout most of its length the gravid uterus becomes greatly kinked and its wall vacuolated.

The more median pair of the excretory canals, the main excretory canals, persists as the excretory system of the gravid segment. This pair of canals (Fig. 9, M.E) becomes greatly expanded, fusing medially at both the anterior and posterior ends of the segment. By their fusion they obliterate the well defined transverse excretory canals of the mature proglottid. The fused longitudinal canals thus form an open vesicle in the anterior and posterior portions of the segment and the cavity of this extends from one segment into the next, passing uninterrupted through the junction between adjacent segments. In neither the whole mount nor the sectioned material are we able to find any trace of the smaller, more lateral pair of excretory canals in the gravid segment.

In sectioned material all of the essential features of the female reproductive system can be distinguished readily. The ovary (Figs. 5, 6 and 9, O) is represented by a bi-lobed, more or less hollow sac. Both of the lateral lobes of the ovary become constricted mesally and the median part arches dorsally and anteriorly from the ventral aspect to about the level of the median plane, forming the isthmus. From the isthmus the oviduct (Fig. 5, OD) arises. It turns posteriorly and ventrally and passes to very near the caudal boundary of the segment before turning anteriorly. The oöcapt (Figs. 5, 6 and 9, OC) is readily seen surrounding the oviduct at the latter's origin from the isthmus. The ascending oviduct (Fig. 9, OD) soon becomes separated into the uterus (U_1) and the vagina (V). Almost at once the uterus receives the common yolk duct (Fig. 5, C.Y.D). From this point the uterus passes anteriorly through an almost straight course, along a somewhat more ventral plane

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Fig. 8. Cross section through expanded uterus (U_2) at junction with the thin-walled, delicate part of the uterus (U_1). Expanded uterus lies more lateral while junction of uteri and vagina lie in midline of the segment. Drawn with aid of camera lucida to same scale as Fig. 5.

Fig. 9. Camera lucida sketch of gravid segment, showing diagrammatic details of the female reproductive system. The ovary and vitellariae are drawn as though they were widely separated. Dorsal view.

Abbreviations

AT—atrium
C—cirrus
C.C—calcareous corpuscle
C.M—circular muscle
C.P—cirrus pouch
CU—cuticle
C.Y.D—common yolk duct
G.P—genital pore
L.E—lateral excretory canal
L.M—longitudinal muscle
M.E—main excretory canal
O—ovary
OC—oöcapt
OD—oviduct
OV—ovum
P—paruterine organ
P.C—paruterine cells
R.M—radial muscle
S.G—shell gland
S.V—seminal vesicle
T—testis
T.E.D—transverse excretory canal
U—uterus
U_1—uterus from oviduct to main part of uterus above the paruterine organ
U_2—uterus anterior to paruterine organ
U.W—uterine wall
V—vagina
V.D—vas deferens
V.E—vas efferens
VIT—vitelline gland
Y.D—yolk duct

than that of the vagina, to a level just anterior to the egg-capsule. Up to this point the uterus is a very delicate, thin-walled tube. Anterior to the egg-capsule the uterus (U_1) suddenly becomes expanded into the egg-storing uterus (U_2). At the point of expansion the uterus gives rise posteriorly to the blind pouch of the egg-capsule and anteriorly to the more opened tube of the egg-storing uterus. In the gravid segment the open part of the uterus contains relatively few ova except in the area adjacent to the egg-capsule. Before the egg-capsule is fully developed the ova are stored temporarily in the expanded uterus (U_2), filling that part of the uterus to capacity. Gradually the ova are crowded from the tubular uterus into the egg-capsule and the expanded uterus undergoes partial atrophy. The wall of the uterus becomes vacuolated, the tube is thrown into a contorted series of kinks, twists and turns, and the lumen is greatly constricted or completely obliterated by the vacuolation of the wall. In the most gravid segment the uterus may become so shrunken that it extends only as far forward as the level of the genital pore.

The egg-capsule is formed as an evagination from that part of the uterus which was embedded in the paruterine cells of the mature segment. Its lumen is derived directly from the lumen of the uterus and the major portion of the wall comes from the paruterine cells. In some segments in our collection the egg-capsule appears to be globular in shape, with a large central cavity, a very short caudal appendage and very thick walls. In other segments from the same chain it appears to be more ovoid in shape, with thinner walls and a longer caudal appendage. Usually the more ovoid the egg-capsule the more pronounced is the caudal appendage. Always the cavity of the capsule is confined to the more globular portion and this is in open communication with the uterus. However as the segment becomes more gravid the opening into the egg-capsule is restricted as the ova are forced into its lumen by the obliteration of the lumen of the expanded uterus. In none of our sections does the cavity of the egg-capsule extend into the caudal appendage although the appendage may be equally as long as the vesicular portion. The appendage may extend as far posteriorly as the level of the ovarian isthmus (Fig. 6, P).

From the point of its origin the vagina passes anteriorly along a plane slightly dorsal to the plane of the uterus (Figs. 5, 6, 7 and 8, V) to the level of the uterine expansion. From this point the vagina follows the expanded uterus anteriorly to a level slightly in advance of the genital pore, remaining throughout this distance closely adherent to the ventral aspect of the uterus. At its most anterior level the vagina turns laterally and dorsally and passes abruptly around the uterus before descending to the genital pore. In passing around the uterus the wall of the vagina becomes thickened and more muscular and the lumen becomes expanded into the cavity which may serve as the seminal receptaculum. The vagina enters the genital atrium from the antero-ventral aspect.

The vitelline glands, like the ovary, are represented in the fully gravid segment by two more or less hollow sacs (Figs. 5 and 9, VIT). These lie in the dorsal aspect of the medullary portion of the segment, partly lateral and posterior to the lobes of the ovary. The vitelline ducts are easily traced in sectioned material.

DISCUSSION

In the present paper we have attempted to give some conception of the details of the anatomy for both the mature and gravid segment of *Mesocestoides variabilis*

Mueller, 1928, from the opossum, *Didelphis virginiana* Kerr. The general features of the anatomy readily adapt themselves to the general plan of the cyclophyllidean cestode but differ from previous conceptions as described for the MESOCESTOIDIDAE in two principal respects: (1) both pair of the excretory canals lie on the same ventro-dorsal plane and (2) the genital pore is dorsal in position.

In standard references on cestode anatomy it is generally stated that the surfaces of the strobila can be designated as dorsal and ventral and that the ovary lies nearest the ventral surface. Usually there are two pairs of excretory canals present and the larger of these is designated as the ventral and the smaller as the dorsal pair.

In our material there are two pairs of longitudinal excretory canals in the mature segment. The larger, more median pair of these is connected by transverse canals and persists throughout the entire strobila. The transverse canals lie in the junction between two adjacent segments and belong to no one segment in particular. In the gravid segment the well defined transverse excretory canals of the mature segment are obliterated by the median expansion of the longitudinal canals. The cavity formed by the expanded longitudinal canals extends from the posterior end of one segment into the anterior end of the next, passing uninterrupted through the junction between the adjoining segments. The smaller, more lateral pair of longitudinal canals do not join by means of transverse canals and even disappear from the segment as the gravid condition is attained. Both pairs of the canals lie on the same dorso-ventral plane in the medullary portion of the segment. In regards to the excretory canals there can be no designation of dorsal and ventral surfaces.

In the material at our disposal the ovary lies nearer to one surface than to the other. Certainly in our material the ovary begins in the area adjacent to the longitudinal muscles of one side and develops the main body of the ovary between that point and the plane of the longitudinal excretory canals, the mid-dorso-ventral plane. Only the ovarian isthmus extends beyond this plane and then only for a sufficient distance for the position to be determined. However the oviduct arises from the isthmus at its most dorsal point. Yet the genital ducts originating from the oviduct swing back to the ovarian side of the segment and only gradually do they resume the more median plane. Therefore, if we can rightfully assume the ovarian side to be the ventral side of the segment, the genital pore is dorsal in position in *M. variabilis* since it opens to the outside through a pore which is situated on the surface opposite that next to which the ovary lies.

SUMMARY

Several incomplete strobilae of a cestode from the opossum, *Didelphis virginiana* Kerr, from Mississippi, have been identified as being identical with *Mesocestoides variabilis* Mueller, 1928. In no instance was a scolex found, although a large number of mature and gravid proglottides is available for study. The material was studied from whole mounts, cross, sagittal and frontal sections.

Attention is drawn to the general topography of the mature and gravid segments, especially to the formation of the egg-capsule and the connections made by the various ducts of the genital systems.

The dorso-ventral axis of the species is discussed from the standpoint of the paired longitudinal excretory canals and the position of the ovary relative to the two surfaces of the segment.

REFERENCES

CAMERON T. W. M. 1935. The cestode genus *Monocercoides* Vaillant. J. Helm. 3: 33–44.

CHANDLER A. C. 1942. *Monocercoides merceri* n. sp. from a lynx, with notes on other North American species of *Mesocestoides*. J. Parasitol. 28: 227–231.

CHANDLER G. B. 1936. Some notes on cestodes from Nebraska. J. Parasitol. 22: 400.

FUHRMANN O. 1928. Ordnung Klasse des Cladus Plathelminthes, Cestoidea. In Handbuch der Zoologie, Kükenthal and Krumbach, pp. 141–416.

HERMAN R. J. C. 1935. *Monocercoides spei* a new species of cestode from the mouse. J. Parasitol. 12: 91–96.

JOYEUX C. AND BAER, J. G. 1942. Recherches sur les cestodes appartenant au genre *Mesocestoides* Vaillant. Bull. Soc. Path. Exot. 25: 993–1010.

MACCALLUM G. A. 1921. Studies in helminthology. Zoopath. 1: 247–248.

MORGAN J. F. 1937. Two new species of the cestode genus *Monocercoides*. Tr. Am. Mic. Soc. 46: 294.

———1938. The genus *Monocercoides* in mammals. Zool. Jahrb. Syst. Abt. 55: 403–418.

———1939. Cestodes of the genus *Monocercoides* from the opossum. Am. Midland Naturalist 12: 81–90.

WITENBERG G. 1934. Studies on the cestode genus *Monocercoides*. Arch. Zool. Italy 20: 447–508.

ZSCHOKKE F. 1900. Recherches sur la structure anatomique et histologique des cestodes. Mem. Inst. Nat., Genevois 19: 1–172.

PHYLLODISTOMUM COATNEYI N. SP., A TREMATODE FROM THE URINARY BLADDER OF *AMBYSTOMA MACULATUM* (SHAW)

F. G. MESERVE

In September, 1938, nineteen adult salamanders were brought to the laboratory. They were collected in the basement of a summer cottage at Bass Lake, Wisconsin, approximately thirty miles east of St. Paul, Minnesota. Two out of nineteen specimens of *Ambystoma maculatum* were infected.

The following description is based on six out of ten specimens. Measurements were made from four specimens which included the largest and smallest animals. Drawings were made with the aid of the camera lucida.

Phyllodistomum coatneyi n. sp.
(Fig. 1.)

Description: Body non-flat unsegmented with posterior part not set off from anterior part. Three times as long as wide with greatest width across the ovarian region approximately half way between the ends. Margins smooth.

Length 1.0–1.9 mm, greatest width 0.7–1.7 mm. Mouth ventral, sub-terminal. Oral sucker 0.301–0.473 mm in width. Acetabulum at posterior end of first third of body length, 0.455–0.630 mm in width, slightly to right of mid-line. Muscular pharynx lacking. Esophagus relatively slender and almost straight, 0.215–0.344 mm in length. Intestinal rami slightly wavy and relatively large in diameter, left somewhat longer than right, 0.103–0.396 mm from posterior end of body.

Longitudinal axis of sex glands somewhat oblique. Testes irregularly and deeply lobed, in posterior third of body region, oblique. Anterior testis to right of mid-line, 0.429–1.229 mm in length by 0.361–0.860 mm in width. Posterior testis approximately in mid-line, 0.137–0.559 mm from posterior end of body. Seminal vesicle broader than long, 0.094–0.258 mm in length by 0.129–0.412 mm in width, in mid-line to left of anterior end of uterus, opening into a short and wide prostate gland. Vas deferens and excretory vesicle not seen in whole mounts.

Ovary to left of mid-line approximately half way between the two ends, deeply lobed, 0.335–0.559 mm in length by 0.258–0.430 mm in width. Vitelline glands to right and anterior to

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The author wishes to give credit to F. C. Wurman, the collector; R. A. Boyce who made the rote mounts; Drs. James E. Lynch and John S. Rankin, Jr. who furnished specimens of related species; Mr. Frank J. Lewis for valuable suggestions; Dr. H. W. Manter who furnished reprints from his private library and Dr. E. W. Price for citations to pertinent literature.

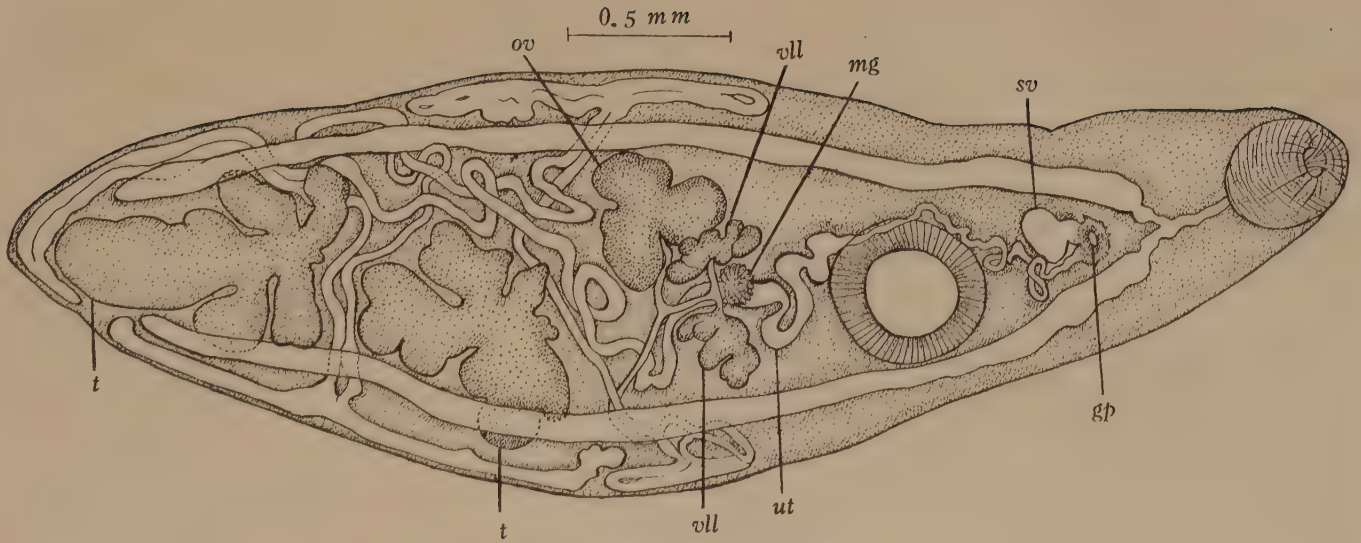

FIG. 1. *Phyllodistomum coatneyi* n. sp.

gp	genital pore	*sv*	seminal vesicle	*ut*	uterus
mg	shell gland	*t*	testis	*vll*	vitelline gland
ov	ovary				

Drawing made with the aid of a camera lucida.

ovary, deeply lobed and slightly oblique, 0.189–0.464 mm in length by 0.129–0.189 mm in width. Vitelline ducts unite between the vitelline glands to form the common vitelline duct which passes posteriorly to join the oviduct from the left and the uterus from the right. Oviduct leaves the ovary on the right side and passes to right and slightly posterior to join the vitelline duct and uterus. Mehlis' gland between vitelline glands and just anterior to vitelline ducts. Genital pore between oral sucker and acetabulum somewhat posterior to bifurcation of esophagus to form the intestinal crura, in mid-ventral line, 0.232–0.387 mm from the acetabulum and 0.774–0.989 mm from the anterior end. Laurer's canal not seen. Seminal receptacle absent.

Uterus passes posterior to right of seminal vesicle by a tortuous path to left of acetabulum. Just posterior to acetabulum it widens considerably, turns obliquely to the right and after several loops it passes almost straight posterior half way between the vitelline glands. It now turns to the right and after passing posterior to the ovary it turns to the left and passes obliquely posterior to the outside of the left intestinal limb opposite the posterior testis where it forms a loop, then turns to the right across the body of the animal at the level of the posterior third of the body between the testes. It then passes posteriorly between the lateral margin of the animal and the right intestinal limb where it forms a loop and passes forward to the level of the posterior end of the ovary. It now passes to the left between the testes forming another posterior loop on the left outside the intestine. Turning back on itself it passes forward and turns left to lateral edge of animal forming a posterior and anterior loop at level of middle third of body. It then turns posteriorly and passes obliquely posterior to the mid-line and then obliquely anterior to the right forming a V posterior to the ovary. It now forms a short posterior and two short anterior loops opposite the ovary on the right side near the lateral edge of the animal. The uterus now passes obliquely anterior to the left where it joins the oviduct to the right of the ovary. Eggs non-operculate 0.021–0.029 mm in length by 0.015–0.020 mm in width containing embryos.

Host: Ambystoma maculatum (Shaw).

Habitat: Urinary bladder.

Locality: Bass Lake, Wisconsin.

Type specimens: Two cotypes will be deposited in the United States National Museum.

P. coatneyi is most like *P. americanum* although there are marked differences. The vitellaria of *P. americanum* are approximately the same size as the ovary whereas in *P. coatneyi* they are only about one half the size of the ovary. The testes of *P. coatneyi* are larger, have more lobes and extend farther forward than in *P. americanum*. In *P. americanum* the uterine loops are more numerous and differ in position, particularly in not extending to the margins of the body; the seminal vesicle is larger and the Mehlis gland is smaller than in *P. coatneyi*. The greatest difference is in the relative shape and size of the eggs which are round and 0.052 by 0.050 mm in *P. americanum* and oval and 0.021–0.029 by 0.015–0.020 mm in *P. coatneyi*.

P. coatneyi is named in honor of Dr. G. R. Coatney of the United States Public Health Service.

References

Braun, Max 1899 Ueber Clinostomum Leidy. Zool. Anz. **22**: 484–493.

Bhalerao, G. D. 1937 Studies on the helminths of India. Trematoda IV. J. Helm. **15**: 97–124.

Lewis, F. J. 1935 The trematode genus *Phyllodistomum* Braun. Tr. Am. Micr. Soc. **54**: 103–117.

Osborn, H. L. 1903 On *Phyllodistomum americanum* n. sp., a parasite in the urinary bladder of Amblystoma tigrinum Green, in Minnesota. Science **17**: 532–533.

———1903 On *Phyllodistomum americanum* (n. sp.) a new bladder distome from *Amblystoma punctatum*. Biol. Bull. **4**: 252–258.

Pande, B. P. 1937 On the morphology and systematic position of a new bladder fluke from an Indian frog. Ann. and Mag. Nat. Hist. **20**: 250–256.

RESEARCH NOTES

A TRAY FOR COLLECTING ANOPHELINE MOSQUITO LARVAE

The larvae of anopheline mosquitoes feed and rest while at the water surface usually in contact with floating or emergent objects. When disturbed, they characteristically swim short distances at the surface or, after swimming downwards a few inches, quickly return to the surface. Because of this behavior, the common practice of collecting larvae by skimming the water in likely breeding spots with a dipper or shallow pan generally gives good results. Frequently, however, larvae of *Anopheles quadrimaculatus,* the important malaria carrier in the southeastern United States, are found among heavy emergent vegetation, brush, or floating debris where surface dipping or skimming is ineffective.

A device now in use in the Georgia Department of Public Health and offering some advantages over the dipper or pan, is a shallow wooden tray, the bottom of which is made of slats. The tray measures about 9 by 16 inches and is about ½ inch deep (see Fig. 1). The slats which

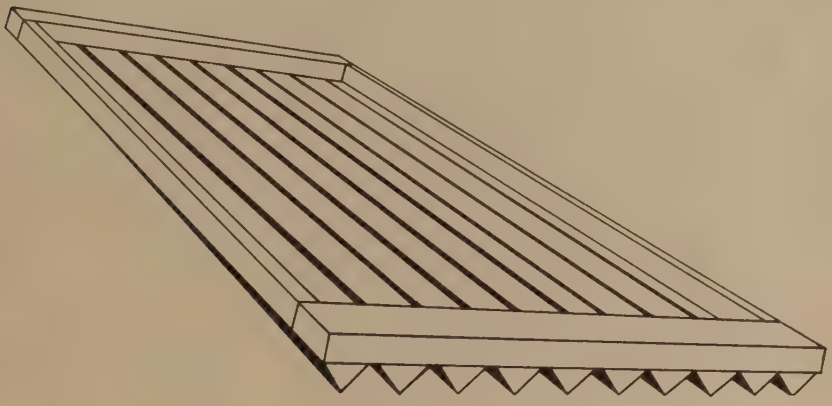

FIG. 1.

are triangular in cross section, are about ¾ inch wide and are set 1/8 to 3/16 of an inch apart. Thus the bottom of the tray is provided with a flat upper surface and a grill-like under surface. The tray is finished with a heavy smooth coat of white enamel.

In use the tray is placed on floating or emergent objects and pressed down, forcing them below the surface. The larvae quickly work out of the submerged mass and pass with the water through the narrow slits into the tray where they become conspicuous against the white background. Then, while the tray is held down by pressing on it with the specimen bottle, the larvae can be collected with a pipette.

Contribution No. 18, from the Division of Malaria and Hookworm Service, Georgia Department of Public Health, Atlanta, Ga.—PAUL C. BEAVER, *Georgia Department of Public Health, Atlanta, Georgia.*

A PHYSALOPTERAN (NEMATODA) FROM THE DOMESTIC PIG

Numerous specimens of immature *Physaloptera* sp. were recovered from the mucosa of a pig stomach near Manhattan, Kansas, during the month of March. None of the other 30 pigs examined was infected with this worm. These specimens, which at first were mistaken for pieces of straw, were deeply embedded in the mucosa of the stomach and only the posterior end was visible. Of some twenty specimens recovered, the range in length was from 3.4 to 6.5 mm. These worms belong to the genus *Physaloptera* as characterized on the basis of dentition by Schultz (1927, Samml. Helminth. Arbeit. Prof. Skrjabin, Moskva, pp. 287–312). The arrangement of papillae, amphids, and the dentition follows the description of Chitwood and Chitwood (1938, An Introduction to Nematology, Sec. I, Part II, p. 61) for this genus. Generic designation on the basis of uteri groupings (Morgan, 1940, J. Parasitol. **26** Suppl: 40) cannot be determined because of the immaturity of the specimens. Likewise specific diagnosis cannot be

attempted. This appears to be the first report in any country of *Physaloptera* sp. from the pig.—W. M. Reid, *Monmouth College, Monmouth, Illinois, and Kansas State College, Manhattan.*

THE TYPE OF *GYROCOELIA MILLIGANI* LINTON, 1927

Identification of certain avian cestodes necessitated better knowledge of *Gyrocoelia milligani*, which was described by Linton (1927, Proc. U. S. Nat. Mus. **70**: 1–75) from a sanderling, *Crocethia alba*. The type material, a single slide, was loaned to me through the courtesy of Dr. Benjamin Schwartz.

The type material consists of five fragments from two or more specimens, representing two distinct genera, which have recently (Fuhrmann, 1936, Ann. Parasitol., **14**: 261–271) been placed in separate families. The fragments are as follows: (a) A short, broad piece of *Gyrocoelia* female strobila containing nine segments. Evidently one of these segments served for Fig. 190 of Linton's paper (op. cit.). The cirrus pouch (cirrus inverted) (Burt [1939, Spol. Zeylonica **21**: 195–208] suspects these structures are actually vaginal pouch and vagina) attains a length of 380 μ. Genital pores alternate irregularly. (b) Another short, broad piece of a *Gyrocoelia* female containing thirteen segments; probably from the same worm as (a), immediately posterior to that fragment. The uterus is plainly circular in several segments, although Linton did not mention this important generic character. Genital pores alternate irregularly. (c) An irregular fragment containing parts of about eleven segments, probably a more anterior piece of the *Gyrocoelia* specimen. (d) A slender fragment containing about twenty segments, but no scolex or evident genitalia. Probably an early piece of the *Gyrocoelia*. (e) Fifteen segments and scolex; a slender, entire specimen of a *Progynotaenia*. The hooks are missing, however, as Linton indicated; the cirri are the only internal organs visible. This specimen, unfortunately, was regarded by Linton as the anterior part of the *Gyrocoelia*, and so figured and described (op. cit.; Figs. 188, 189 and p. 59). But the genital pores are regularly alternate, the suckers are prominent, and the worn, everted cirrus has the characteristic (for *Progynotaenia*) bell-shaped tip, as shown in Linton's Fig. 189. My measurements for this specimen are: Rostellum 66 μ in diameter; sucker 122 μ in diameter; scolex 215 μ in diameter; cirrus pouch 200 to 238 μ long. Definite specific identification seems impossible without rostellar hooks. Fuhrmann (1932, Mem. Univ. Neuchatel **8**: 1–381; 173), evidently on the basis of Linton's figure, transferred *milligani* to the genus *Progynotaenia*.

Much of Linton's description was based upon sectioned material, which I have not seen, but which was not part of the designated type. Nevertheless, it may be presumed that this material was *Gyrocoelia*, as Baer pointed out (1940, Parasitology **32**: 174–197), because Linton stated that the cirrus passed between the lateral excretory vessels.

Baer (op. cit.) recently referred material from a kildeer, *Oxyechus vociferus*, from Antigua in the West Indies to Linton's species. Because his material and description are excellent, the best taxonomic procedure seems to be as follows: the type of *Gyrocoelia milligani* Linton, 1927, is hereby restricted to those two adjacent fragments described above as (a) and (b) and measuring 4 mm × 1.2 mm and 6 mm × 1.3 mm on slide 7892, U. S. N. M. Helm. Coll. The publication of Baer (op. cit.) is taken as the first revision; the specimens referred to therein are assumed identical with the lectotype thus designated. If future extensive examination of the type host, *Crocethia alba*, proves that the *Gyrocoelia* occurring therein is not conspecific with Baer's specimens, redefinition of the species will be necessary.—J. Dan Webster, *Rice Institute, Houston, Texas.*

DISPHARYNX SPIRALIS IN GOLDEN AND RING-NECKED PHEASANTS IN NEW YORK

During October, 1941, we received for autopsy a male and a female golden pheasant (*Chrysolophus pictus*) from a game farm near Mexico, New York (Oswego County). The male bird was found to be infected with several proventricular worms, which were identified as *Dispharynx spiralis*. We believe this is a new host record for this parasite.

In July, 1942, three wild ring-necked pheasant chicks (*Phasianus colchicus*) from eastern Long Island, New York (Suffolk County), were examined. Two of these were parasitized with *D. spiralis*. Recently Madsen (1941, J. Parasitol. **27**: 29–34) reported this nematode from ring-necked pheasant chicks in Denmark. He reported that its presence in partridges was a new European host record, but did not claim a new host record for the pheasant, since he cited Cram (1928, U. S. Dept. Agric. Tech. Bull. **49**: 1–9) as previously reporting it from pheasants.

It seems possible, however, that Cram's 1928 record represents a lapsus of some sort, since pheasants are not included in her previous list (1927, U. S. Nat. Mus. Bull. **140**: 239) or her later paper (1931, U. S. Dept. Agric. Tech. Bull. **227**: 1–27). Cuvillier (1937, Rabot. Gel'm.-

Skrjabin: 99), who had access to Cram's records, listed seven galliform hosts for *Dispharynx spiralis* but the pheasant was not among them.—Frans C. Goble and E. L. Cheatum, *Research Center, New York State Conservation Department, Delmar, New York.*

A NOTE ON THE LIFE CYCLE OF *AUSTRALORBIS GLABRATUS* (SAY, 1818) PILSBRY, 1934, A SNAIL INTERMEDIATE HOST OF *SCHISTOSOMA MANSONI*

Shortly after hatching from eggs, fourteen specimens of the snail, *Australorbis glabratus* (syn. *Planorbis guadeloupensis* Sowerby, 1822), were isolated, one to a battery jar which contained three liters of water and some aquatic plants. The water of these cultures had a pH between 7.5 and 8.5. It was found that water samples collected in places where there were large populations of these snails in the field also had a low alkalinity. The aquatic plant used in the cultures was either *Naias guadeloupensis* or *Eichornia azurea* which species are commonly found in the natural habitat of *A. glabratus* in Venezuela. Small pieces of fresh lettuce were added to the cultures twice a week and the water was renewed as frequently as was necessary. The battery jars were covered with glass plates in order to reduce evaporation. The cultures were maintained in a place supplied with ample indirect sunlight and here the temperatures of the water of the cultures ranged from 20° to 29° C. Since this snail is disc-shaped and grows in a concentric fashion, the diameter was used as an index of its growth. Table 1 includes some observations which were made on the fourteen snails during their first year of life in the laboratory.

The data in the table emphasize the variations in the development and egg-production of these fourteen snails which came originally from only three different groups of eggs. Oviposition, in the snails began from 79 to 190 days following hatching. The diameter of the snails at that time ranged from 6 to 13 millimeters. The total number of eggs deposited by each snail during the first 12 months of life varied from 55 to 1045. At the end of the first year the snails were still growing and laying eggs in the cultures. The presence of one or the other species of plants mentioned did not seem to effect the growth or egg-production of the snails. There did not seem to be any correlation between the number of eggs deposited by each snail and the age of the snail at its first oviposition.

As described by Hoffman and Faust (1934, Puerto Rico J. Pub. Health and Trop. Med. 9: 228–282), it was found that *A. glabratus* laid groups of from 1 to 30 eggs embedded in a translucent albuminoid substance and each egg had a length of about 1 millimeter. With the temperatures ranging from 20° to 29° C in the laboratory, it took from 8 to 12 days for the eggs to develop and hatch. In three different tests it was found that 64% of the eggs hatched.

Table 1.—*The development and egg-production of the snail,* Australorbis glabratus, *during its first year of life*

Snail	First oviposition		Total number of eggs produced	Diameter at end of first year (mm)
	Age (days)	Diameter (mm)		
1	79	8.0	1045	15.0
2	81	7.0	885	14.5
3	101	7.3	158	15.5
4	139	8.0	374	16.0
5	139	9.0	960	15.0
6	145	9.5	644	16.0
7	149	6.0	367	13.5
8	156	8.0	399	17.0
9	156	10.0	451	16.0
10	172	13.0	57	18.0
11	172	9.0	828	15.5
12	175	10.5	162	15.5
13	178	14.0	182	18.0
14	190	13.0	55	19.0

The observations made in the laboratory and in the field, indicate that the life cycle of this snail continues throughout the year in Venezuela. By comparing the average diameter of the *A. glabratus* in the year-old cultures in the laboratory with the diameter of the largest specimens of this species which were found in the field, the duration of the life of *A. glabratus* may be estimated as one year and a half to two years.—George W. Luttermoser, *Instituto Nacional de Higiene, Caracas, Venezuela.*

ALBERT HASSALL (1862–1942)

IN MEMORIAM

Albert Hassall (1862–1942)

On September 18, 1942, Dr. Albert Hassall, parasitologist and bibliographer, passed away at Bangor, Me., as the result of a heart attack following a surgical operation, and was buried September 23, at Collington, Md. He is survived by one sister, two sons and three daughters.

Albert Hassall, the only son of Major Thomas and Elizabeth Hassall, was born February 12, 1862, in Woolwich, Kent, England. After receiving his early education in private schools, he entered the Royal Veterinary College in London and was graduated as a Member of the Royal College of Veterinary Surgeons from that institution in 1886. While in London he had as one of his professors the late Dr. T. Spencer Cobbold, at that time England's most distinguished parasitologist, whose influence over this young veterinary student was largely responsible for his future career. After his graduation, Hassall's father wished him to enter the Colonial Service; this did not appeal to Albert, so he came to the United States in 1887 and almost immediately entered the infant Bureau of Animal Industry. He was appointed veterinary inspector and assigned to duty to assist in the eradication of contagious pleuropneumonia, a destructive disease of cattle that had gained a foothold in this country a few years previously. On April 30, 1890, he entered veterinary practice in Baltimore, but Cobbold's influence had been too profound and on March 7, 1891, he reentered the government service as assistant to the late Dr. Cooper Curtice, who was the Bureau's first parasitologist. Following the resignation on May 31, 1891, of Dr. Curtice and the appointment on June 3 of the same year of Dr. Ch. Wardell Stiles, in charge of the Zoological Laboratory, Hassall was Stiles' principal assistant until November 29, 1902, when he was transferred to Chicago, Ill., for duty in the Meat Inspection Service. Upon the resignation of Stiles to become Chief of the Division of Zoology of the Hygienic Laboratory of the United States Public Health and Marine Hospital Service, now the National Institute of Health, and of the appointment of the late Dr. B. H. Ransom to succeed him in the Bureau, Hassall returned to Washington and on April 14, 1904, was assigned to duty in the Zoological Division. At his own request he was assigned to bibliographic work, continuing the Index-Catalogue which he had started several years previously. Hassall served from 1905 to 1910 as assistant in zoology and from 1910 to 1924 as assistant zoologist under Ransom, and from 1928 to 1932 as senior zoologist and Assistant Chief of the Zoological Division under the late Dr. M. C. Hall. In 1932, having reached retirement age, Hassall was given a two-year extension to continue his work, but owing to the Economy Act he was retired on June 30 of that year. For several months Hassall continued to work without compensation and on July 16, 1934, he was given the title of Collaborator in order that he might have official status in the Bureau and to permit the publication under his name of the Index-Catalogue of Medical and Veterinary Zoology which was being reissued.

Hassall's scientific interests date from his student days under Cobbold. In his earlier years he was an indefatigable collector and his extensive collection is deposited in the U. S. National Museum, forming a substantial part of the nucleus of the Helminthological Collections of that institution.

The Index-Catalogue of Medical and Veterinary Zoology, with which he was most closely identified, was started by him shortly after he became Stiles' assistant. One of his duties was to look up references to the literature for his chief, and since these references were, at that time, difficult to secure, he conceived the idea of saving the reference cards so that they might be readily available for future use. With the passage of time the accumulation of references was expanded to meet the needs of the increased staff of the laboratory and then to meet the requirements of a growing and expanding science of parasitology.

Hassall published only about 15 papers under his own name, the greater part of his work appearing under the joint authorship of Stiles and Hassall. This was partly due to his lack of desire for personal glory and partly because of a custom prevailing at the time he entered the government service which decreed that heads of departments in educational institutions and in government divisions and offices were, ipso facto, senior authors of all papers from their respective units. Hassall, however, persistently refused to occupy any rank lower than that of junior author or to bask in reflected glory of big name authors, maintaining that he would "never play third fiddle in any orchestra." As a result of this attitude, a few monographic papers which embodied a large amount of his work do not bear his name. The joint authorship of Stiles and Hassall ended with the publication in 1929 of Bulletin 152 of the Hygienic Laboratory, U. S. Public Health Service, entitled "Key-Catalogue of Parasites Reported for Primates (Monkeys and Lemurs) with Their Possible Public Health Importance." At this time the Damon and Pythias of parasitology agreed to cease joint publication, with the understanding that the Key-Catalogue series was to be published by Stiles and the Index-Catalogue series by Hassall.

For the most part Hassall's published work was that of compilation, a type of endeavor that does not gain the plaudits of the thoughtless, or of the neophyte having his initial fling in the scientific world, or of those who have failed to acquire wisdom with age, even though it saves these individuals incalculable hours of labor. Furthermore, Hassall's characteristic thoroughness assures the users of his compilations that, so far as humanly possible, the information contained in them is complete and reliable.

Some idea of the thoroughness of his work may be gained from the following verses written by the late Dr. N. A. Cobb and presented at a birthday dinner given in honor of Dr. Hassall in 1932:

The Recording Angel (Dr. Albert Hassall)

When I meet the recording angel
Of the mighty B. A. I.
My sins rise up before me
And smite me hip and thigh.
For I know there's no escaping
His well known, eagle eye.
He spots my every error
And intends to till I die.

And he writes them in his Files,
Inscribes them in his Files.

If I have two MS species
Of the same generic form,
And because I'm absent-minded
Or my brain is in a storm,
I name them in terms identic
For my old friend Dr. Dorm,

It's, "What the hell you doing?"—
Or something just as "warm."
So this escapes his Files,
Thanks be, escapes his Files.

But if I rediscover
A form from the misty past
And mistakenly rename it,
I never hear the last
Of how I've bunged the works up
With the "wrenches" I have cast,
"At your abysmal ignorance
The whole world stands aghast."
For he'll have it in his Files,
He'll have it in his Files.

If I named a species *"minor"*
In the long, long, long ago,
And after time's gone flying
What seems an age or so,—
I apply it to a brother species,
I quaff the bitterest woe!
It's "Cobb, your case is hopeless,
I'd make my will and go!"
He'll have it in his Files,
He'll have it in his Files.

And when I land in Tophet
I shall hear it relayed down
From the land I left behind me,
As I sizzle and I brown;—
"There's one thing I'd forgotten,
That genus from beyond the town,—
The name you went and gave it
Is an *adjective,* not a noun."
It's up there in his Files,
It's up there in his Files.

As a result of his bibliographic work, Hassall became an authority on the subject of zoological nomenclature. He had a retentive memory and his accumulated knowledge and experience were always available to his associates and to all who genuinely sought information. He was a thoroughgoing rugged individualist who wore no man's collar and scraped no man's boots; he was a stickler for truth and admitted no compromise for the sake of expediency. In spite of his ruggedness, he was a kindly gentleman and a sincere friend whose greatest pleasure was in helping others.

Hassall took little interest in scientific societies, owing largely to the fact that he was a farmer during his free hours. However, he was an early member of the Helminthological Society of Washington, served as its president in 1921–22, and was elected to life membership in 1931.

For his distinguished service and in recognition of his contributions to science and veterinary medicine, the Royal College of Veterinary Surgeons in London awarded to Dr. Hassall the Steel Memorial Medal in 1922, given for pre-eminent work.

May he rest in peace in the congenial company of those whose names he immortalized in the Index-Catalogue of Medical and Veterinary Zoology.—E. W. Price and G. Dikmans, *Zoological Division, U. S. Bureau of Animal Industry.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Thirty-first Council Meeting, New York City

January 9, 1943

The meeting of the Council of the American Society of Parasitologists was called to order by Dr. Henry E. Meleney, President of the Society, at 2:00 PM, January 9, 1943, in the offices of the Department of Preventive Medicine, New York University, 341 East Twenty-fifth Street, New York City. The following members of the Council were present: D. L. Augustine, R. M. Cable, J. T. Culbertson, R. W. Glaser, H. E. Meleney, G. F. Otto, L. E. Rozeboom, N. R. Stoll, and H. W. Stunkard.

I. Reports of Officers

1. *Secretary (J. T. Culbertson):* As of December 31, 1942, there were 534 persons on the membership roll of the Society, of which 463 lived within and 71 lived outside continental United States. Of the total number on the roll, 437 were members in good standing and 99 were delinquent for from one to three years. Of those in good standing, 396 lived within and 41 outside continental United States. Fifty new members were elected during the year, of which number 45 lived within and 5 without the United States. Four members died during the year: Dr. Robert Hegner of Baltimore, Maryland, Dr. William James of Ancon, Republic of Panama, Dr. Marcus W. Lyon, Jr., of South Bend, Indiana, and Dr. Winfield C. Sweet of La Paz, Bolivia.

Upon motion the Secretary's report was accepted and placed on file.

2. *Treasurer (L. E. Rozeboom):* For the fiscal year 1942 (Dec. 2, 1941 to Dec. 7, 1942):

Receipts

Balance on hand, Dec. 2, 1942		$1805.83	
Collected during current year			
Member dues applying 1942	$ 975.31		
" " " 1943	933.95		
Advance dues	31.50		
		$1940.76	
Subscriptions applying 1942	1734.56		
" " 1943	360.00		
Advance subscriptions	4.50		
		2099.06	
Back nos. and vols. sold	373.55		
25-volume index sales	180.60		
Contributions from members	224.00		
Advertisements	65.50		
Author charges	123.77		
Miscellaneous	49.52		
		5056.76	
Loan from Princeton Fund		400.00	
			$7262.59

Expenditures

Printing 25-vol. index, balance	$ 754.78		
" Journal, 1941 account	754.14		
" " 1942 "	2532.07		
		4040.99	
Expenses in office of Chm. Ed. Comm.		111.43	
" " " " Secretary		162.44	
" " " " Treasurer		395.34	
Miscellaneous		27.24	
			-4737.44
Balance on hand Dec. 7, 1942			$2525.15

Upon motion the Treasurer's report was accepted, subject to audit, and placed on file.

Council voted that the Treasurer's report be published in summary form, covering all business transacted during the fiscal year 1942, but omitting all allocations. Council also voted that the Treasurer be instructed to account in reports for future years for all moneys on hand, for all business transacted, and for the cash balance at the end of the then current fiscal year, giving due regard for essential itemization.

II. Reports of Committees

1. *Custodian of the Secretarial Fund (N. R. Stoll):* For the fiscal year Dec. 1, 1941 to Dec. 1, 1942:

Total liquid assets	$ 475.79
Book value, subsidiary assets	816.67
Total	1292.46
Total, preceding year	1276.77
Increase, total assets current year	15.69

Upon motion, the report was accepted, subject to audit, and placed on file.

2. *Chairman of the Editorial Committee (N. R. Stoll):* During 1942, the six regular numbers of Volume 28 of the Journal of Parasitology containing 506 pages and the December Supplement containing 50 pages were issued on schedule. The regular numbers contained 85 articles and research notes and 8 miscellaneous reports. The interval between "received for publication" and date published averaged 11.0 months in 1942 for regular articles (10.7 in 1941), and 10.3 months for research notes (9.1 in 1941). It was noted that manuscripts submitted in the years 1938 to 1942 totalled for the successive years, respectively, 84, 95, 107, 100, and 79.

Three complications in publishing the journal were mentioned: (1) the loss of members and subscribers in part through the government prohibition of export to certain countries; (2) the requirement that each issue in proof form be cleared through the Office of Censorship; and (3) possible shortages of materials.

Certain changes in format begin with the February, 1943, issue for a two-year experimental period: (1) the type page will be slightly enlarged; and (2) blank or partly blank pages will when possible be omitted. Beginning with the next volume, authors will be charged for pages in excess of 10, instead of 20 as at present.

Upon motion, the report was accepted and placed on file.

3. *Auditing Committee (D. L. Augustine and R. M. Cable):* The Auditing Committee approved the reports of the Treasurer and of the Custodian of the Secretarial Fund.

Upon motion the report of the Auditing Committee was accepted and placed on file.

III. Reports of Representatives of the Society

1. *Representative to the Union of American Biological Societies (G. L. Graham):* Prof. E. G. Butler is the new president of the Union of American Biological Societies. Plans are under way to expand the activities of the Union, particularly with regard to advising with respect to the increasing number of applicants for Latin-American fellowships to this country. It is recommended that "the American Society of Parasitologists adopt the policy and procedure of voting a regular annual contribution to the Union of sufficient amount to be of real assistance in conducting their affairs and that said contribution be made without request on the part of the officers of the Union." By vote of Council the report of the representative was accepted, with the reservation that no financial contribution be made at this time for the support of the Union or of Biological Abstracts.

IV. New Business

1. *New Members:* Council voted membership in the Society upon four applicants: Lester L. Bissinger, Department of Zoology, University of Minnesota, Minneapolis, Minnesota; Margarita B. Hollis, Instituto de Biologia, Chapultepec, Mexico, D.F.; C. Clayton Hoff, Department of Zoology, Quincy College, Quincy, Illinois; and Morton C. Kahn, Department of Preventive Medicine, Cornell University Medical College, New York City.

2. *Next Annual Meeting:* The selection of a meeting place for the Society in 1943 was deferred by vote of Council until more information with respect to such a meeting be available.

It was suggested by G. F. Otto that if war conditions should prevent the meeting of the entire Council at the end of 1943, the Society's business be conducted by mail, possibly following a meeting of those Council members living in the New York area.

3. *Program Committee:* Council voted to establish an Advisory Committee on Program and Abstracts consisting of the Secretary (Chairman), the Chairman of the Editorial Committee, and the President of the Society.

4. *New Business Related to* JOURNAL OF PARASITOLOGY:

(1) *Sales of the Journal to American Library Association's "Committee on Libraries in War Areas":* Council voted to approve future sales of the JOURNAL at four dollars per volume to the Committee on Libraries in War Areas.

(2) *Reprints of Abstracts in December Supplement:* Council voted that recommendation be made to the Editorial Committee of the JOURNAL that no reprints of individual abstracts printed in the Supplement be made available.

(3) *Insurance of Journals in Storage:* Council voted to authorize the Treasurer to insure journals now stored at the Johns Hopkins University School of Hygiene and Public Health against damage by fire, water, or other hazards including those related to the war. Details of the procurement of this insurance were left to the discretion of the Treasurer.

(4) *Custodian of the Journal Stock:* Council voted to authorize the Treasurer to appoint a Custodian of the Journal stock, should the necessity arise.

(5) *Miscellaneous:* Council agreed with the suggestion of the Chairman of the Editorial Committee that the basic issue of the JOURNAL be raised from 1000 to 1100 copies.

5. *Election of Officers, etc.:* Following extended discussion, Council voted (seven votes for and one against) that present officers and members of Council be continued in office until the next meeting of the Society. Accordingly, officers for 1943, or until the Society's next meeting, are: *President,* H. E. Meleney, *Vice-president,* R. W. Glaser, *Secretary,* J. T. Culbertson, and *Treasurer,* L. E. Rozeboom. Likewise, E. W. Price and E. R. Becker, who were originally scheduled for retirement from Council, will continue as Council members.

The Nominating Committee (W. W. Cort, Chairman) for the Selection of a New Editorial Committee for the JOURNAL recommended the following: H. W. Stunkard (Chairman), W. A. Riley, and D. H. Wenrich. The new Editorial Committee would serve for five years assuming responsibility with Volume 30. Council voted to accept the recommendations of the Nominating Committee.

For the three annual vacancies on the Editorial Board of the JOURNAL, Council voted to elect R. W. Glaser, B. G. Chitwood, and P. P. Levine as successors to C. B. Philip, A. C. Chandler, and E. E. Tyzzer, respectively, whose terms had expired.

President H. E. Meleney appointed E. W. Price Chairman of the Nomenclature Committee to succeed P. D. Harwood, who had resigned. The President reappointed the present representatives of the Society on the Council of the American Association for the Advancement of Science, and to the Union of American Biological Societies.

The meeting was adjourned at 9:45 PM. From 5:45 until 7:30 PM the meeting had been recessed in order that the members of Council entertain the President of the Society at dinner in the Gramercy Park Hotel, New York City.

Respectfully submitted,

JAMES T. CULBERTSON, *Secretary.*